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A minisatellite DNA marker for discriminating between European and North American Atlantic salmon (*Salmo salar*)

John B. Taggart, Eric Verspoor, Paul T. Galvin, Paloma Morán, and Andrew Ferguson

Abstract: A highly discriminatory and practical nuclear DNA genetic marker that can distinguish between Atlantic salmon (*Salmo salar*) of European and North American origin is described. Screening of 2847 European and 247 North American Atlantic salmon from much of its geographic range for variability at a minisatellite locus, *Ssa*-A45/2/2, revealed the continental stocks to be almost fixed for two different-sized, easily discernable alleles. Virtually all European Atlantic salmon were homozygous for a 3.00-kb allele (frequency > 0.999), while a smaller 2.77-kb allele (frequency = 0.946) predominated in all North American populations. Whereas the 2.77-kb allele was found exclusively in North American salmon, an allele indistinguishable in size from the 3.00-kb European diagnostic allele was also observed at low frequency (0.036) in North American fish. Eight other continent-specific rare alleles (highest frequency = 0.006) were also observed. The results suggest that little, if any, natural gene flow occurs between the two continental groups of Atlantic salmon.

Résumé : Nous décrivons un marqueur génétique de l'ADN nucléaire hautement discriminant et pratique qui permet de distinguer les saumons atlantiques (*Salmo salar*) selon leur origine européenne ou nord-américaine. L'examen de 2847 saumons européens et 247 saumons nord-américains provenant d'une bonne partie de leur aire géographique, qui portait sur la variabilité à un locus de minisatellite, *Ssa*-A45/2/2, a révélé que les stocks continentaux étaient presque fixes en ce qui concerne deux allèles de taille différente et faciles à distinguer. Pratiquement tous les saumons atlantiques européens étaient homozygotes pour un allèle de 3,00 kb (fréquence > 0,990), tandis qu'un allèle plus petit de 2,77 kb (fréquence = 0,946) prédominait chez toutes les populations d'Amérique du Nord. Tandis que l'allèle de 2,77 kb se retrouvait exclusivement chez les saumons nord-américains, un allèle impossible de distinguer par la taille de l'allèle de 3,00 kb servant au diagnostic des saumons européens a aussi été observé à une faible fréquence (0,036) chez les poissons d'Amérique du Nord. Huit autres allèles rares spécifiques à un continent (fréquence maximale = 0,006) ont aussi été observés. Ces résultats permettent de penser qu'il n'y a pratiquement aucun flux génique naturel entre les deux groupes continentaux de saumons atlantiques.

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Introduction

Genetic differentiation between North American and European populations of Atlantic salmon (*Salmo salar*) has been investigated over the past three decades. This has been stimulated both by fundamental evolutionary interest and practical management considerations, particularly the search for a means to identify salmon caught in mixed-stock oceanic fisheries to continent of origin.

While early karyological studies (e.g., Boothroyd 1959; Rees 1967) had documented differences in chromosome arm number in North American and European salmon, the significance of these separate papers was not immediately recognized. Around the same period initial electrophoretic studies (of blood protein polymorphisms) provided direct

evidence of distinctive genetic differences between North American and European stocks (Nyman 1966; Payne et al. 1971; Nyman and Pippy 1972). Later, from more comprehensive surveys of many enzyme-coding loci, Ståhl (1987) and Verspoor (1988) confirmed the presence of a major genetic dichotomy between western and eastern Atlantic populations. However, no fixed allele differences between the two groups have yet been found at any locus. While discrimination of stocks in a mixed-fisheries sample has proved possible through protein analyses (e.g., Fairbairn 1979; Payne 1980; Verspoor 1988) the need for invasive sampling and frozen tissue storage has proven to be a serious obstacle to its routine implementation.

More recently, attention has turned to appraisal of both mitochondrial (mt) and nuclear DNA variability as possible sources of more discriminatory and less labile genetic markers for this task. Bermingham et al. (1991) reported the presence of three different composite mtDNA haplotypes among 115 Atlantic salmon. All North American fish (n = 65) had the same haplotype, which was also present in one European salmon. The other two haplotypes were found exclusively in all other salmon of European origin screened (n = 49). The usefulness of this approach in providing diagnostic continental stock markers has yet to be fully ascertained. Additional studies have since detected European-type haplotypes (up to 75% in some samples) in Atlantic salmon populations from Newfoundland, Nova Scotia, and Quebec (Birt et al. 1991; McVeigh et al. 1991).

Similarly, a promising nuclear DNA marker was described by Cutler et al. (1991). Preliminary screening with a probe specific for ribosomal RNA genes detected diagnostic phenotypic differences in Southern blot autoradiographic patterns between Atlantic salmon DNA samples from Newfoundland (n = 33) and Scotland (n = 27). However, with very few individuals (from relatively restricted geographic ranges) examined to date, and the underlying genetic basis of the observed polymorphism yet to be clearly established, the utility of this marker too needs further verification.

In this paper the discriminatory potential and practical applicability of another nuclear DNA marker are assessed. During preliminary characterization studies of a panel of salmonid-derived single-locus minisatellite DNA probes (Taggart and Ferguson 1990; Prodöhl et al. 1994*a*, 1994*b*; Taggart et al. 1995) one particular locus was found to be almost completely invariant among four European populations of Atlantic salmon. Later screening of a few North American samples showed most individuals to be homozygous for an alternative allele. As a result of ongoing population surveys this locus has now been typed in 2847 and 247 salmon of European and North American origin, respectively, the results of this extensive screening being summarized here.

Materials and methods

Samples

Atlantic salmon from 53 river drainages (Tables 1 and 2), representing a large part of the geographic range of the species, were collected by electrofishing, netting, or fixed traps. In most cases, samples comprised juvenile fish (parr, smolts). Both anadromous and nonanadromous stocks from North America were included in the study (Table 2). Tissue samples (skeletal muscle, liver, or adipose fin) were stored frozen (−20 to −70°C) or preserved in 95% ethanol prior to use.

Population screening

In most cases total genomic DNA was isolated using a simplified phenol–chloroform method (Taggart et al. 1992). Standard protocols for agarose gel electrophoresis (3 μg *Pal*I (or *Hae*III) digested DNA per sample; 25 cm long gels), Southern blotting (onto Hybond N membrane; Amersham International plc), and isotopic labelling of probe (random priming) were followed. Prehybridization and hybridization conditions were 4× SSPE (0.72 M NaCl, 40 mM NaH_2PO_4, 4.0 mM ethylenediaminetetraacetic acid (EDTA) Na_2; pH 7.7), 0.5% dried milk, 1% sodium dodecyl sulphate (SDS), and 6% polyethylene glycol (PEG) at 65°C (modified from Dalgleish 1987). Sonicated salmon sperm DNA (Sigma D-9156) was added, as competitor, to the hybridization mix at a concentration of 15 $\mu g \cdot mL^{-1}$. Following overnight hybridization, membranes were washed at high stringency, i.e., 0.2× SSC (2× SSC is 0.3 M NaCl, 0.03 M trisodium citrate; pH 7.0), 0.1% SDS, 65°C, 2× 30 min, and autoradiographed for 1–3 days at −70°C in the presence of one intensifying screen.

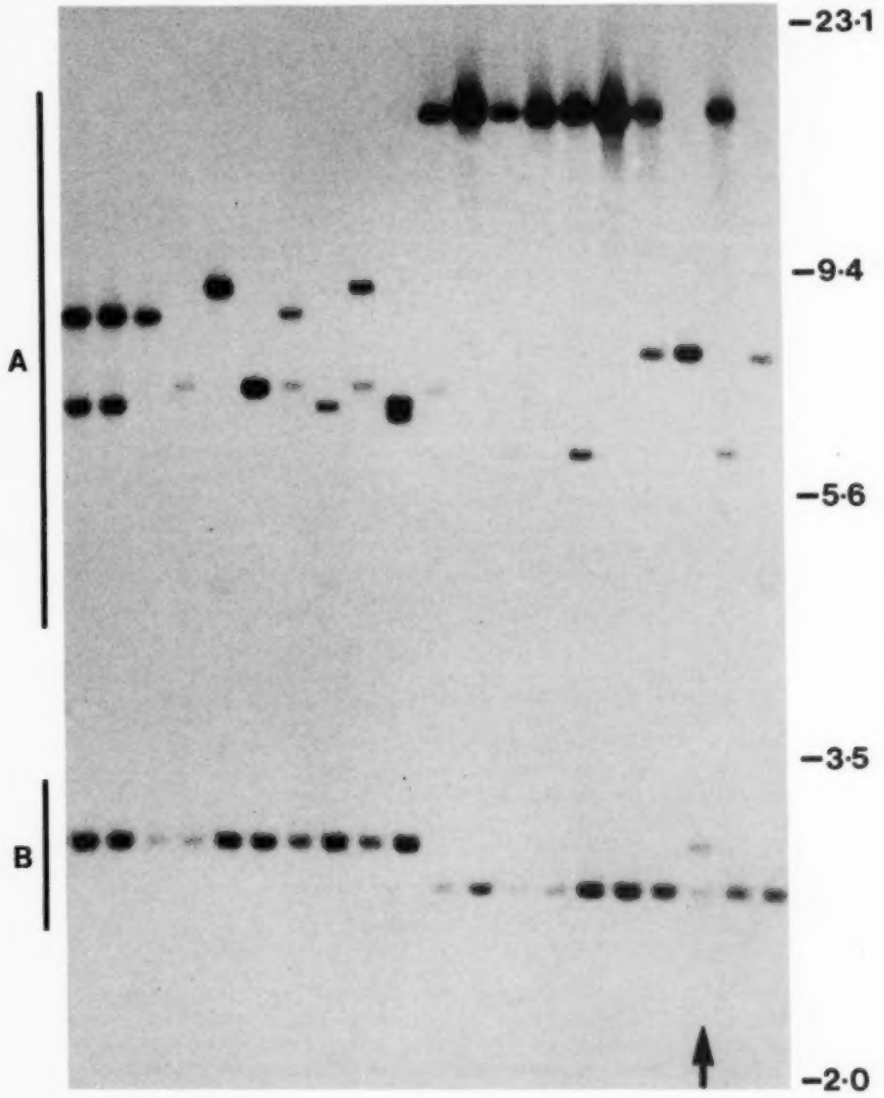
The minisatellite probe derived from Atlantic salmon that was used in this study was pSsa-A45/2. The isolation and characterization of this probe, and locus terminology, have been detailed elsewhere (Taggart et al. 1995). Allele sizes were estimated with reference to internal DNA standard markers (Taggart and Ferguson 1994).

Results

When membranes were probed with pSsa-A45/2, autoradiographs revealed distinct allelic fragments for two separate loci, termed *Ssa*-A45/2/1 and *Ssa*-A45/2/2 (Figs. 1A and 1B). The former, with alleles ranging from 4.5 to 18.0 kilobase pairs (kb), was highly polymorphic in both the European and North American Atlantic salmon samples. The larger the allele the more intense was the resulting signal. The size range of alleles largely overlapped between the two groups, though alleles larger than 14 kb were confined to North American fish (combined frequency for these large alleles = 0.23). Both the high number (at least 30) and nearly continuous distribution in size (between 6 and 8 kb) of the majority of alleles prevented unambiguous scoring of most individuals on the large scale involved in this study. Disomic inheritance at this locus has been previously demonstrated (Taggart et al. 1995).

In contrast, the allelic fragments for *Ssa*-A45/2/2 were much smaller (2.5–3.2 kb) and the locus itself was much less variable, with most individuals (99.1%) being homozygotes. Occasional two-banded (heterozygous) phenotypes (Fig. 1B, Tables 1 and 2) were observed. DNA profiles of representative homozygous and heterozygous samples digested with an alternative restriction enzyme (*Mbo*1) were essentially the same as their *Pal*1-digested counterparts (data not shown). The number and relative size of fragments were identical, though all *Mbo*1 fragments were

Fig. 1. DNA profiles from *Pal*1-digested samples of 20 Atlantic salmon (from left: 1–10, European; 11–20, North American) detected with minisatellite probe pSsa-A45/2. Solid lines (left side) indicate the size range of observed allelic fragments at *Ssa*-A45/2/1 (A) and *Ssa*-A45/2/2 (B). The size scale (right side) is given in kilobase pairs (kb). An uncommon *Ssa*-A45/2/2 heterozygote (2.77/3.00 kb) from the Conne River, Newfoundland, is shown by the arrow. Differences in band intensities among *Ssa*-A45/2/2 homozygote phenotypes can be solely attributed to variations in the amount of DNA loaded in each lane.



about 0.5 kb smaller than their *Pal*1-digested equivalents. This is consistent with the observed polymorphism being due to a variable number of tandem repeats at a minisatellite locus, rather than being the result of restriction site polymorphisms at either *Ssa*-A45/2/1 or *Ssa*-A45/2/2. Confirmatory inheritance studies for *Ssa*-A45/2/2 have not yet been possible because of the rarity of informative (heterozygous) parents.

The population survey revealed major allele and genotype frequency differences at *Ssa*-A45/2/2 between Atlantic salmon of European and North American origin (Tables 1 and 2). Virtually all European salmon were homozygous for a 3.00-kb allele (overall frequency > 0.999) while a smaller 2.77-kb allele (overall frequency = 0.946) predominated in all North American populations (Fig. 1). While the latter was found exclusively in North American

Table 1. Source, numbers, and *Ssa*-A45/2/2 genotype scoring of European Atlantic salmon samples.

Region	River	Type[a]	No.	*Ssa*-A45/2/2 genotype			
				3.00/3.00	3.00/2.91	3.00/2.86	3.00/2.74
Iceland	Fljótha	J	10	10	—	—	—
	Sog	J	10	10	—	—	—
Norway	Dale	J	13	13	—	—	—
	Imsa	J	10	10	—	—	—
	Vosso	J	7	7	—	—	—
Sweden (Baltic)	Torne	J	5	5	—	—	—
	Kalix	J	5	5	—	—	—
	Dahle älv	J	10	10	—	—	—
Great Britain	Polla	J	10	10	—	—	—
	Shin	J	40	40	—	—	—
	Dee	A	511	510	1	—	—
	Loth	J	5	4	1	—	—
	Nith	J	10	10	—	—	—
	Troutbeck[b]	J	45	45	—	—	—
	Leven[b]	J	86	86	—	—	—
	Rothay[b]	J	102	102	—	—	—
	Teign	J	10	10	—	—	—
Ireland	Glenarm	J	28	28	—	—	—
	Rathcool	J	20	20	—	—	—
	Glen	J	9	9	—	—	—
	Glenaniff	J	10	10	—	—	—
	Delphi	J	11	10	—	—	1
	Corrib	J	29	28	—	1	—
	Burrischoole	J	50	50	—	—	—
	Shannon	J	1050	1050	—	—	—
France	d'Orloron	J	10	10	—	—	—
	Douron	J	10	10	—	—	—
	Nivelle	A+J	41	41	—	—	—
Spain	Cares	A+J	234	234	—	—	—
	Esva	A+J	250	250	—	—	—
	Landro	A	8	8	—	—	—
	Mandeo	A+J	59	59	—	—	—
	Masma	A	23	23	—	—	—
	Minio	A	26	26	—	—	—
	Ulla	A+J	90	90	—	—	—
Totals			2847	2843	2	1	1

[a]Life history stage: A, adult (returning sea migrants); J, juvenile (parr or smolt).
[b]S.E. Hartley, Department of Biological and Molecular Sciences, University of Stirling, U.K., unpublished data.

salmon, an allele identical in size to the European diagnostic one (i.e., 3.00 kb) was also observed, albeit at low frequency (0.036), in 9 of the 17 North American populations surveyed. Only a single North American parr, from the Margaree River, had a genotype (3.00/3.00 kb) indistinguishable from the sample of Atlantic salmon of European origin. Eight other very low frequency alleles were scored (three in European and five in North American populations). These too were specific to only one or other continental group (Tables 1 and 2).

Five European river drainages were intensively surveyed (sample sizes > 100 individuals; Table 1). There was no indication of the North American diagnostic allele being present even at a very low level in any of these. While this has resulted in an overall skewed sampling regime, the overrepresentation of these five largest samples (75% of all European samples analyzed) has a relatively minor effect on calculated allele and genotype frequencies. For example, exclusion of these five drainages only decreases the estimated frequency of the diagnostic 3.00-kb allele from >0.999 to >0.997.

Discussion

An ideal genetic marker is one in which the populations or stocks in question are fixed for alternative alleles (or haplotypes), allowing the unambiguous identification of all individuals. This situation is rarely found among naturally

Table 2. Source, numbers, and *Ssa*-A45/2/2 genotype scoring for North American Atlantic salmon samples.

Region	River	Type[a]	No.	*Ssa*-A45/2/2 genotype							
				2.77/2.77	2.77/3.00	3.00/3.00	2.77/3.18	2.77/3.13	2.77/2.96	2.77/2.61	2.77/2.56
Newfoundland	Gander – Little Gull[b,c]	M+J	18	15	2	—	—	—	—	—	1
	Big Triangle Pond[c]	M+J	6	6	—	—	—	—	—	—	—
	Aquaforte[c]	M+J	12	12	—	—	—	—	—	—	—
	Bristol Cove[c]	M+J	10	10	—	—	—	—	—	—	—
	Long Beach[c]	M+J	9	9	—	—	—	—	—	—	—
	Conne	J	18	17	1	—	—	—	—	—	—
New Brunswick	Nepisiquit	J	9	7	1	—	—	1	—	—	—
	Saint John	A	10	8	2	—	—	—	—	—	—
	SW Miramichi	A+J	10	9	—	—	—	—	1	—	—
Nova Scotia	LaHave	A	10	10	—	—	—	—	—	—	—
	Margaree	A	9	7	1	1	—	—	—	—	—
Maine	Horricks (hatchery strain)	A	10	7	—	—	3	—	—	—	—
	Dennys	J	32	27	4	—	—	—	—	1	—
	Sheepscot	J	20	20	—	—	—	—	—	—	—
	Ducktrap	J	24	22	2	—	—	—	—	—	—
	Machias	J	20	18	2	—	—	—	—	—	—
	Narraguagus	J	20	19	1	—	—	—	—	—	—
Total			247	223	16	1	3	1	1	1	1

[a]Life history stage: A, adult (returning sea migrants); M, mature (nonanadromous stocks); J, juvenile (parr or smolt).
[b]Mixture of anadromous (Gander) and nonanadromous (Little Gull) populations.
[c]Nonanadromous populations.

occurring fish populations (Pella and Milner 1987). However, as reported here, the polymorphic minisatellite locus *Ssa*-A45/2/2 does closely approach this goal. Both allele and genotype frequency differences between the two continental stocks at this locus are more extreme than those reported for any protein polymorphism (e.g., Ståhl 1987; Verspoor 1988). On the basis of the composite genotype frequencies at the four most discriminatory isozyme loci (requiring both skeletal muscle and liver tissue sampling) Verspoor (1988) estimated that 97.6% of North American and 97% of European Atlantic salmon could be correctly assigned from a sample made up of an equal mixture of the two stock groups. Using a similar model, and even assuming a frequency for the 3.00-kb allele of 0.1 among North American anadromous stocks (more than twice that actually observed in this study), 99.5% of individuals would be correctly assigned to continent of origin through screening of this single locus.

Reflecting our current research programmes, the majority of Atlantic salmon screened in this study were of European origin, and comprised fish from throughout a large part of the species range in this area. The total absence of the common 2.77-kb North American allele among these fish provides convincing evidence for the lack of gene flow from western Atlantic to eastern Atlantic populations. This tends to confirm traditional physical tagging studies where few intercontinental migrations have been recorded. During the 10-year period 1974–1983, only four salmon tagged in Canada were recovered in Europe, and six tagged in Europe were found in North American waters (Reddin et al. 1984). Such documented occurrences, however, give no measure of the reproductive success of migrants. Some multilocus isozyme analyses have suggested that Atlantic salmon from Baltic Sea populations are genetically distinct from the rest of eastern Atlantic stocks (Ståhl 1987; Verspoor 1988). This dichotomy was not detected by *Ssa*-A45/2/2 screening, nor was it present in a more extensive analysis of protein data carried out by Verspoor (1994).

Fewer North American populations (and individuals) were examined. In spite of this, *Ssa*-A45/2/2 was more variable in these populations (seven alleles segregating; observed heterozygosity = 0.093) than in European stocks (four alleles segregating; observed heterozygosity < 0.002). The same 2.77-kb allele predominated in all populations. This situation is very likely to be representative of the North American group as a whole. Clearly, though, much more extensive screening, including populations from the extreme northern parts of the range (e.g., Labrador), would be desirable both to confirm this view and to investigate further the occurrence and distribution of the less common alleles. While European-type mtDNA haplotypes have only been reported among more northerly populations (Birt et al. 1991; McVeigh et al. 1991) the 3.00-kb allele, equivalent in size to the European diagnostic one, was found throughout the North American geographic range examined. Whether this allele was endemic to the ancestral founders of present day North American populations, has independently arisen by mutation, or represents continuous or intermittent gene flow from European stocks (through natural straying and (or) more recent aquacultural introductions) remains to be established. Behnke (1980) has hypothesized that Atlantic salmon from both European and North American glacial refugia colonized Newfoundland following the end of the last Ice Age. A more extensive screening of North American landlocked Atlantic salmon populations may prove informative in this respect.

In addition to being highly discriminatory, *Ssa*-A45/2/2 variation is relatively straightforward to examine. As with mitochondrial analyses genomic DNA can be readily extracted from salmonid tissues preserved in alcohol at room temperature (Taggart et al. 1992). Only a small biopsy sample (e.g., fin clip fragment) is required. Routine screening of minisatellite DNA polymorphisms is possible at both reasonable cost and effort. Although radioactive labelling was used in this study, nonisotopic protocols could be readily substituted, as reported elsewhere for salmonid minisatellite studies (Bentzen and Wright 1993; Stevens et al. 1993). Furthermore, by cloning and sequencing the diagnostic DNA fragments it should be possible to develop a simpler polymerase chain reaction (PCR) based methodology to screen for this variation. This would open up the possibility of detailed historical studies. Many thousands of dried scales samples (which are suitable for PCR-based screening) from both river and former mixed-seas fisheries are archived. A comparison with DNA sequences of other minisatellite loci, which generally show much higher levels of variability (Prodöhl et al. 1994*b*; Taggart et al. 1995), may also shed light upon the molecular mechanisms involved in the generation of length mutations at these loci.

A range of applications for this nuclear DNA genetic marker can be envisaged. Despite commercial oceanic fisheries being temporarily suspended at present, there is a continuing need for scientific evaluation of Atlantic salmon high seas stocks. Screening for *Ssa*-A45/2/2 variation may also be of value in determining the fate of historic intercontinental introductions. Recent aquacultural practice has also seen the importation into European fish farms of out of season Atlantic salmon ova of North American origin, from hatcheries in the southern hemisphere (Scottish Office Agriculture and Fisheries Department 1994). Genetic monitoring of these imports both within the confines of rearing facilities and among natural populations, should escapes occur or be suspected, would be feasible. Regarding these latter applications, potential hybridization and introgression with native stocks would be detectable following successful spawning of either male or female introduced fish. Being maternally inherited, analogous mtDNA markers would be insensitive to either mature parr or adult male genetic contributions. Screening of *Ssa*-A45/2/2 and mtDNA variation in tandem could ascertain the relative contributions of non-native male and female salmon where hybridization occurs. Similarly, more detailed surveys of both *Ssa*-A45/2/2 and mtDNA variation among North American populations would undoubtedly provide further insights into the pattern of colonization of Atlantic salmon within this continental region (Verspoor 1994). Such work would also help to clarify whether (or to what minor extent) natural gene flow from European to North American stocks occurs.

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Effect of habitat selection and behavior on vulnerability to predation of introduced fish

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Abstract: Losses from resident predators can be an important source of mortality for introduced fish, but may vary among species. I compared vulnerability between muskellunge (*Esox masquinongy*) and walleye (*Stizostedion vitreum*) to predation by largemouth bass (*Micropterus salmoides*). In pool experiments ($N = 51$) with simulated vegetation, muskellunge were more susceptible to predation than walleye. Habitat selection explained some of these differences as walleye spent more time in the simulated vegetation and associated with the substrate than muskellunge. Expectations from pool experiments were confirmed in reservoirs stocked with two size groups of walleye ($N = 8$ introductions) and esocids ($N = 20$). Walleye were less susceptible to largemouth bass predation for both small (mean 14% of stocked fish) and large (mean 0%) size groups than were small (mean 36%) and large (mean 21%) esocids of three taxa. For muskellunge only, walleye were less vulnerable to predation for large size groups, but not for small ones. Predation from largemouth bass should be a more important source of poststocking mortality for esocids than for walleye in lakes and reservoirs. Largemouth bass population demographics, specific to each system and year, should be considered more carefully in determining where esocids should be introduced than for percids.

Résumé : Les pertes causées par les prédateurs résidants peuvent être une importante cause de mortalité chez les poissons introduits, mais varient souvent d'une espèce à l'autre. J'ai comparé la vulnérabilité maskinongé (*Esox masquinongy*) et du doré jaune (*Stizostedion vitreum*) à la prédation par l'achigan à grande bouche (*Micropterus salmoides*). Dans des expériences menées en bassin ($N = 51$) avec de la végétation simulée, le maskinongé était plus vulnérable à la prédation que le doré. Le choix de l'habitat expliquait une partie des différences, car le doré passait plus de temps dans la végétation simulée et à proximité du substrat que le maskinongé. Les résultats des expériences en bassin ont été confirmés dans des réservoirs où on a ensemencé deux groupes de taille de doré ($N = 8$ introductions) et d'ésocidés ($N = 20$). Le doré était moins vulnérable à la prédation par l'achigan à grande bouche tant pour le groupe de petite taille (moyenne 14% des poissons déversés) que pour le groupe de grande taille (moyenne 0%) que les ésocidés de petite taille (moyenne 36%) et de grande taille (moyenne 21%) appartenant à trois taxons. Par rapport au maskinongé seulement, le doré était moins vulnérable à la prédation dans les groupes de grande taille, mais non dans les groupes de petite taille. La prédation par l'achigan à grande bouche doit donc être une source plus importante de mortalité post-repeuplement pour les ésocidés que pour le doré dans les lacs et réservoirs. Il serait bon d'examiner la démographie des populations d'achigan à grande bouche, spécifiquement pour chaque système et chaque année, de façon plus attentive dans le cas des ésocidés que dans celui des percidés, en vue du repeuplement.
[Traduit par la Rédaction]

Introduction

Predation can determine species composition in natural communities, affecting both diversity and abundance of prey (Macan 1977; Peckarsky and Dodson 1980; Crowder and Cooper 1982; Morin 1984). Similarly, predators may also influence the success of introduced species. Predation is an important factor determining survival of introduced sport fish, including esocids (Stein et al. 1981; Wahl and Stein 1989), walleye (*Stizostedion vitreum*) (Santucci and Wahl 1993), and channel catfish (*Ictalurus punctatus*) (Krummrich and Heidinger 1973). Size influences predation rates for all species, with larger fish being less vulnerable than smaller ones.

Behavior and habitat use can vary among introduced species and may influence predation risk (Werner et al. 1983; Kotler 1984; Mittelbach 1984; Savino and Stein 1989*b*). Distributions, both spatially and temporally, of prey and predators can affect relative vulnerability to predation

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(Noble 1986). Other aspects of behavior, such as schooling and evasive tactics, can also influence vulnerability in fishes (Neill and Cullen 1974; Webb 1986; Wahl and Stein 1988; Savino and Stein 1989*a*). As a result, each introduced species may experience different levels of mortality from resident predators.

Effective management requires an understanding of the potential impacts of predators on the success of a species introduction. However, information on predator susceptibility and causes of differences among species is limited. In this study, I compared the susceptibilities of stocked fishes to predation by largemouth bass (*Micropterus salmoides*), an important piscivore in many lakes and reservoirs. Risk to predation was compared between esocids and walleye, two groups of important introduced sport fish, in laboratory and field experiments. I also examined differential behavior and habitat selection between the species and evaluated their influence on relative susceptibility to predation.

Materials and methods

Laboratory experiments

To compare susceptibility to predation between walleye and muskellunge (*Esox masquinongy*), experiments were conducted in circular pools (2.0 m diameter, 0.5 m deep) at water temperatures ranging from 18 to 23°C and with a photoperiod of 14 h light : 10 h dark. Conditions were similar to those described by Wahl and Stein (1989), with half the pool containing simulated aquatic vegetation (250 stems/m^2) made from polypropylene line (0.5 m long, 4 mm diameter), whereas the other half contained no simulated vegetation. Line sections were fastened to wire mesh, covered with gravel, and extended to the water surface. The density of the simulated vegetation was within that observed for lakes and reservoirs (Savino and Stein 1982).

Two of each prey species (75–175 mm in total length), matched in size (within 5 mm), were added to a pool 24 h before an experiment. The location of walleye and muskellunge (upper or lower 0.25 m of water column) and habitat selection (open or vegetated portion of pool) were recorded for 30 min before introduction of a single largemouth bass. A total of 51 experiments were completed. In 30 of these experiments, prey associated with the substrate (<5 cm from the bottom) were also recorded. The location of walleye and muskellunge was recorded every 2 min (N = 15 observations/experiment); mean values for each experiment were compared between species. Largemouth bass (250–350 mm) starved for 24 h were released into the pool from a holding compartment. Prey lengths were in a size range readily consumed by largemouth bass (30–50% of predator length). Six largemouth bass were each used in 7–15 experiments.

Following release of the largemouth bass, the number of attacks and captures (Webb and Skadsen 1980; Savino and Stein 1982; Moody et al. 1983; Wahl and Stein 1989) on each prey species and in each habitat type was recorded. Capture efficiency was the number of captures divided by the number of strikes in each experiment. Habitat use and distance from the largemouth bass when follows began were recorded for each walleye and muskellunge. Flight distances, defined as the closest distance that a prey will allow a predator to approach before moving (Wahl and Stein 1989), were recorded in the same subset of 30 experiments described above. Distances were estimated to the nearest 5 cm with grids marked on the bottom of the tank with colored gravel. The behaviors of prey followed by largemouth bass were compared with those of prey not followed. Experiments ended when a largemouth bass captured a walleye or muskellunge.

Each experiment was a replicate for analyses of walleye and muskellunge behavior (N = 30 or 51 depending upon behavior), whereas each largemouth bass was the experimental unit for examination of predator behavior (N = 6). Arcsine transformations were used on percentage data to avoid problems associated with the variance being a function of the mean (Steel and Torrie 1980). Except where indicated, statistical analyses were either one- or two-way analysis of variance (ANOVA) and Tukey's multiple comparisons (Montgomery 1984; SAS Institute Inc. 1985).

Field experiments

To evaluate predictions from laboratory pool experiments, I summarized literature values of losses to largemouth bass predation following stocking for walleye (Santucci and Wahl 1993) and esocids (Stein et al. 1981; Carline et al. 1986; Wahl and Stein 1989). Estimates of mortality for esocids included muskellunge as well as northern pike (*Esox lucius*) and tiger muskellunge (*E. masquinongy* × *E. lucius*) (Table 1). To standardize the effects of stocking size, comparisons were made within two 30-mm size classes with data for both taxonomic groups, one from 130 to 160 mm and a second from 190 to 220 mm. These data from the literature were supplemented by results from two stockings of walleye and six stockings of esocids in Illinois reservoirs. Rearing techniques varied by reservoir stocking and included both intensively and extensively cultured fish, as well as fish reared on either pellets or minnows. For all stockings, fish were individually counted and sorted to be uniform in size (95% confidence limits generally <5 mm) and a sample (N = 100) measured and weighed the day before each stocking. Fish were transported to the reservoirs in aerated tanks with 0.5% salt and tempered to within 5°C of reservoir temperatures to reduce mortality associated with stocking (Mather and Wahl 1989).

Largemouth bass were captured by electroshocking along the entire perimeter of each reservoir on 3 or 4 days in the first week following stocking and weekly thereafter through 30 days following stocking. Largemouth bass were measured in length (nearest 1 mm), weighed (nearest 1 g), and fin clipped, their stomach contents were recovered by pulsed gastric lavage (Foster 1977) or by using clear acrylic tubes (Van Den Avyle and Roussel 1980), and then they were released. Stocked fish recovered from largemouth bass stomachs could not be confused with resident young-of-year fish as no natural reproduction occurred in the reservoirs.

To estimate the number of stocked fish eaten on each day, I divided the number recovered from stomachs by the number of predators examined and then multiplied this proportion by the estimated number of largemouth bass. Values between sampling dates were estimated by linear

Table 1. Estimates of number eaten (%) by largemouth bass for two size groups of walleye (WE) and three taxa of esocids (tiger muskellunge, TM; northern pike, NP; muskellunge, MU) after introduction into Illinois and Ohio reservoirs.

			Stocked fish			Largemouth bass	
Reservoir	Year	Area (ha)	Taxon	No./ ha	Estimated no. eaten (%)	Population estimate (95% C.I.)	Number examined
Size class 130–160 mm							
Ridge	1987[a]	6	WE	65	12	545 (327–965)	21
Ridge	1988[a]	6	WE	60	7	380 (248–608)	104
Ridge	1989[a]	6	WE	69	23	712 (501–1047)	46
Ridge	1990[a]	6	WE	42	28	274 (199–388)	38
Ridge	1992[d]	6	WE	65	2	240 (182–340)	30
Bird	1980[c]	0.8	TM	250	100	118 (75–186)	30
Worthington	1981[c]	2	TM	225	0	(—)	—
Worthington	1981[c]	2	TM	110	31	24 (—)	—
Worthington	1982[c]	2	TM	113	72	149 (—)	—
Kokosing	1984[b]	46	TM	25	54	1758 (1349–2520)	425
Madison	1985[b]	40	TM	25	40	930 (821–1072)	758
Kokosing	1984[b]	46	NP	25	27	1758 (1349–2520)	425
Madison	1985[b]	40	NP	25	35	930 (821–1072)	758
Kokosing	1984[b]	46	MU	25	9	1758 (1349–2520)	425
Madison	1985[b]	40	MU	25	23	930 (821–1072)	758
Pierce	1990[d]	65	MU	20	8	149 (104–224)	113
Size class 190–220 mm							
Ridge	1987[a]	6	WE	21	0	57 (—)	6
Ridge	1988[a]	6	WE	26	0	16 (6–39)	5
Ridge	1990[a]	6	WE	46	0	74 (44–131)	9
Ridge	1991[d]	6	WE	19	0	0	0
Acton	1982[c]	253	TM	9	0	4 (—)	—
North	1985[b]	89	TM	24	1	1030 (782–1509)	283
Paradise	1990[d]	69	TM	18	28	263 (209–331)	208
Paris	1990[d]	67	TM	38	90	919 (829–1018)	486
North	1985[b]	89	NP	24	2	1030 (782–1509)	283
North	1985[b]	89	MU	24	2	1030 (782–1509)	283
George	1990[d]	69	MU	17	26	501 (385–652)	279
Pierce	1990[d]	65	MU	16	17	149 (104–224)	54
George	1992[d]	69	MU	8	21	477 (266–970)	81

Note: Schnabel population estimates and the number examined after each stocking for largemouth bass included the minimum size capable of ingesting the smallest stocked fish. —, indicates data not available.

[a]Santucci and Wahl (1993).

[b]Wahl and Stein (1989).

[c]Carline et al. (1986).

[d]The present study.

extrapolation (Wahl and Stein 1989). Abundance of largemouth bass was determined using Schnabel mark–recapture procedures (Ricker 1975). Only largemouth bass capable of ingesting stocked fish were included in abundance estimates (Table 1). Maximum prey/predator length ratios were similar in the current study to those observed in previous reservoir studies for both walleye (0.57; Santucci and Wahl 1993) and esocids (0.75; Carline et al. 1986; Wahl and Stein 1989). The lengths of the smallest stocked fish in each reservoir were divided by these ratios to determine the sizes of largemouth bass included in each population estimate. Daily estimates of the numbers eaten by largemouth bass were summed to estimate the total number of stocked fish lost to predation (Wahl and Stein 1989; Santucci and Wahl 1993).

Results

Laboratory experiments

Habitat selection and location in the water column differed for walleye and muskellunge. Both prey species spent the majority (>65%) of time in the vegetated portion of

Fig. 1. Comparison of habitat selection and location in the water column for walleye and muskellunge in laboratory pools (2 m diameter); half of each pool contained simulated vegetation. Values are the proportion of time spent in the lower 0.25 m, associated with the substrate (<5 cm), and in the vegetation during 30-min observations (2-min intervals) before release of a largemouth bass. Vertical bars are 95% confidence intervals.

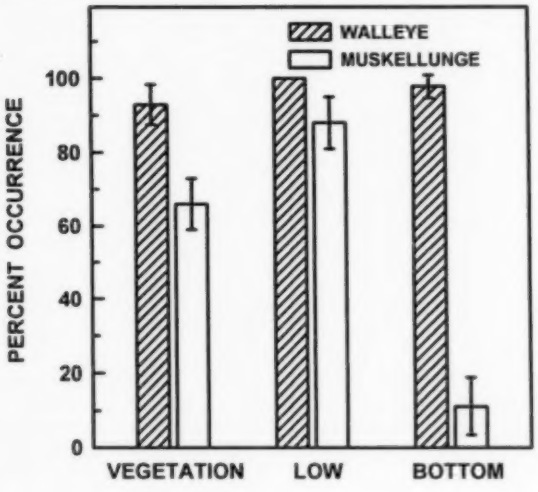
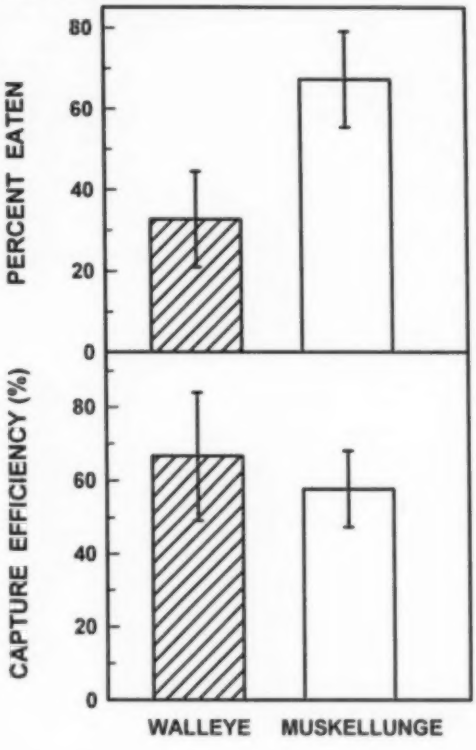


Fig. 2. Number consumed and capture efficiency (captures per strike) for largemouth bass ($N = 6$; 7–15 replicates per fish) feeding on walleye and muskellunge in laboratory pools. Vertical bars are 95% confidence intervals.



the pool, but muskellunge spent more time in the open than walleye (Fig. 1; ANOVA, $P < 0.0001$). Walleye stayed in the lower half of the water column without exception, whereas muskellunge occasionally approached the surface (Fig. 1; ANOVA, $P = 0.0001$). The two species also differed in the amount of time associated with the substrate. Walleye were almost always on the bottom, whereas muskellunge were suspended in the water column, away from the bottom (Fig. 1; ANOVA, $P < 0.0001$).

Largemouth bass consumed more muskellunge than walleye in laboratory experiments. No differences were observed among individual largemouth bass in the number of each prey captured (ANOVA, df = 5, $P = 0.85$). Comparing across largemouth bass, more muskellunge were attacked per experiment (1.84 ± 0.68; mean ± 95% confidence interval) than walleye (0.78 ± 0.4; ANOVA, $P = 0.03$), but capture efficiencies (mean captures per strike) were similar for the two prey (Fig. 2; ANOVA, $P = 0.33$). As a result, more muskellunge were eaten (69%) than walleye (31%; Fig. 2; ANOVA, df = 1, $P = 0.03$).

The behavior of the largemouth bass and of the two prey species appeared to influence predation success. For both prey, largemouth bass struck in equal proportions throughout the water column and between habitats (ANOVA, $P > 0.06$), but captured more prey high in the water column (Table 2; ANOVA, $P = 0.009$) and in the vegetation ($P < 0.001$). No interaction existed between the main effects of species and habitat choice for either number of strikes or captures (ANOVA, $P > 0.89$). However, a significant interaction was observed between species and water column positioning (Table 2). As expected on the basis of time spent in each location, more captures by largemouth bass on walleye occurred low rather than high in the water column (ANOVA, $P < 0.001$) whereas more strikes and captures on muskellunge occurred high in the water column (ANOVA, $P < 0.001$). In contrast to number of strikes and captures, capture efficiency did not differ among either species, locations, or habitats (Table 2; ANOVA, $P > 0.05$), except no captures were made high in the water column for walleye. Similarly, follow distances by largemouth bass did not differ between habitats and species (ANOVA, $P = 0.14$).

Antipredatory behavior of muskellunge and walleye differed. Mean flight distances were similar for muskellunge (25 cm) and walleye (16 cm; ANOVA, $P = 0.09$), but only a few walleye fled from largemouth bass. I also recorded the number of times each species failed to flee as a largemouth bass approached within 30 cm. Walleye remained motionless more often (60%) than did muskellunge (23%; chi-square, $P < 0.006$) as largemouth bass passed over them.

Field experiments

In eight reservoir experiments conducted in Illinois, more esocids were recovered from largemouth bass stomachs

Table 2. Comparison of largemouth bass predatory behavior on walleye (WE) and muskellunge (MU) in laboratory pool experiments ($N = 51$).

Largemouth bass behavior	Species	Location		Habitat	
		High	Low	Vegetation	Open
No. of strikes	WE	0.03±0.1*z*	0.6±0.4*z*	0.5±0.3*ab*	0.2±0.2*b*
	MU	1.4±0.7*y*	0.3±0.4*z*	1.0±0.7*a*	0.7±0.3*ab*
No. of captures	WE	0*z*	0.3±0.1*y*	0.2±0.1*a*	0.1±0.1*b*
	MU	0.6±0.1*x*	0.1±0.1*z*	0.4±0.1*c*	0.3±0.1*a*
Capture efficiency	WE	0*z*	0.68±0.4*y*	0.66±0.4*a*	0.32±0.4*a*
	MU	0.48±0.2*yz*	0.44±0.5*yz*	0.54±0.3*a*	0.41±0.1*a*

Note: Observations are means ± 95% confidence intervals per experiment for either location in the water column (high or low) or habitat (vegetated or open). Six largemouth bass were used in these experiments. Values within or between species followed by the same letter do not differ (ANOVA, $P > 0.05$).

than walleye (see Table 1). Most (>90%) consumption of stocked fish by largemouth bass occurred within 7 days. On the basis of estimates of the number of largemouth bass capable of ingesting esocids (Table 1) and daily estimates of the number of stocked fish eaten, substantial proportions of esocids (range 8–90% of the number stocked), but low percentages of walleye (0–2%) were eaten by largemouth bass (Table 1). Low numbers of largemouth bass of a size able to consume the large size group of walleye were present in Ridge Lake in 1991, but not in previous years (Santucci and Wahl 1993). Overwinter mortality apparently reduced the number of large sizes of largemouth bass.

These estimates of predation by largemouth bass in Illinois reservoirs were combined with estimates from the literature. For 29 reservoir stockings of two size groups of walleye ($N = 9$) and esocids ($N = 20$; Table 1), esocids were more vulnerable to largemouth bass predation than walleye (Fig. 3; ANOVA, $P = 0.02$). This relationship was consistent across both size groups; however, size also influenced losses as small walleye and esocids suffered higher losses than large fish (Fig. 3; ANOVA, $P = 0.04$). Restricting the comparison to muskellunge, walleye were again less vulnerable to predation (ANOVA, $P = 0.002$), but not for both size groups. Small walleye (14%) and muskellunge (13%) did not differ in losses to predation (ANOVA, $P = 0.99$), but larger muskellunge (17%) suffered higher losses than larger walleye (0%; ANOVA, $P = 0.002$; Table 1).

Discussion

Walleye were less vulnerable to largemouth bass predation than muskellunge in both laboratory and field experiments. Because muskellunge are less susceptible to predation than either northern pike or tiger muskellunge (Wahl and Stein 1989), losses of walleye will be lower than for all three esocids. Behavior of largemouth bass and of the two prey observed in the laboratory probably influenced differential vulnerability to predation observed in the field.

Lack of differences in capture efficiency or follow distances between walleye and muskellunge differ from previous studies that found either capture efficiency (related to morphology and swimming speed) or antipredatory behavior to explain differential vulnerability among cyprinid, centrarchid, and clupeid prey species (Webb and Skadsen 1980; Wahl and Stein 1988; Savino and Stein 1989*a*). High capture efficiencies and short follow distances suggest that avoidance behavior is low in muskellunge and walleye. Antipredatory behaviors may be reduced in species that pass through narrow windows of vulnerability, and habitat choice may more strongly influence predation. Differences in habitat use affect predation risk in other species (Werner et al. 1983; Kotler 1984; Mittelbach 1984; Wahl and Stein 1989). Both walleye and muskellunge spent the majority of their time in vegetated habitats; consequently, the number of strikes and captures there were high even though habitat heterogeneity reduces predation rates by piscivores (Nyberg 1971; Mittelbach 1984; Savino and Stein 1982). In contrast, both species also spent the majority of their time low in the water column and foraging rates should have been greater there if prey location was the only important factor. More strikes and captures on muskellunge occurred high in the water column, and previous studies have shown that largemouth bass prefer to forage in these areas (Savino and Stein 1989*a*). Although muskellunge spent a relatively small portion of their time high in the water column, they did spend more time off the bottom than walleye, and it appears that this habitat choice increased their vulnerability to predation.

In the laboratory, largemouth bass are more likely to attack prey that are closer or have greater motion (Howick and O'Brien 1983). Though distances maintained from largemouth bass and flight distances did not differ between species, walleye failed to flee more often than muskellunge. Behaviors affecting predation in the laboratory may not always be important in the field where predators and prey are not confined by pool walls. For some species, decreased motion in the field may actually increase vulnerability by allowing a searching predator to approach within striking distance (Wahl and Stein 1989). For walleye, lack of movement and selection of a position lower in the water column may have interacted to reduce predation mortality.

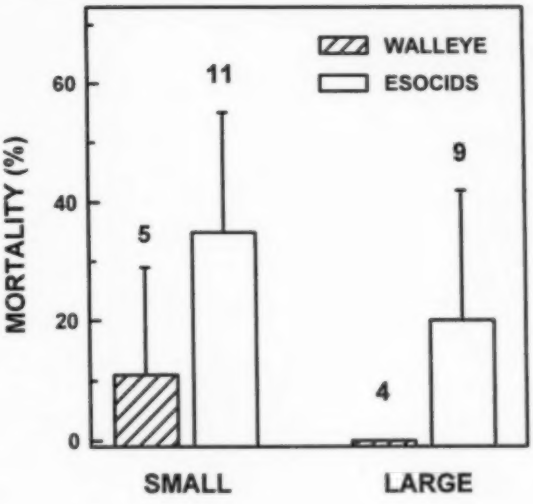
The differences in habitat selection I observed between species in the laboratory were confirmed in previous field

observations (Johnson et al. 1988). In laboratory pools, walleye spent more time associated with the substrate and in the vegetation than muskellunge. The presence of the other species may have influenced these habitat choices; however, Wahl and Stein (1989) found that esocid behavior was not affected by the presence of other taxa in similar pool experiments. As in the laboratory, walleye in the field are generally found near the bottom, inshore early in the year, but offshore during summer (Rawson 1957; Forney 1974; Johnson et al. 1988). Walleye are often associated with structure such as rock or gravel substrate (Johnson 1969: Johnson et al. 1988), which may explain the high use of simulated vegetation and proximity to the substrate in the laboratory. In contrast to walleye, esocids are usually found suspended in littoral, vegetated habitats (Diana et al. 1977; Chapman and MacKay 1984; Wahl and Stein 1989).

Estimates of predatory mortality were highly variable and may have been influenced by several factors including rearing technique, size and timing of stocking, as well as characteristics of individual impoundments. Rearing method differed among species, with tiger muskellunge being fed a pelleted diet. Experience feeding on minnows during rearing has few direct effects on foraging ability (Wahl et al. 1995), but can indirectly increase survival and reduce vulnerability to predation in esocids (Szendrey and Wahl 1995). However, the comparison between muskellunge and walleye was not affected by rearing technique as both were fed minnows in extensive ponds. Size and timing of stocking have been suggested frequently as influencing predation on both walleye (Santucci and Wahl 1993) and esocids (Stein et al. 1981; Carline et al. 1986; Wahl and Stein 1989). Additional reservoir stockings and analyses in this study confirm that predatory losses are lower for larger size-classes of both species. The number of largemouth bass in a population capable of ingesting them is lower and cooler water temperatures reduce food consumption rates by largemouth bass (Rice and Cochran 1984). Estimates of losses to predation assume that largemouth bass stomach contents represent 1 day of feeding, a reasonable assumption given the water temperatures and associated evacuation rates for largemouth bass (Beamish 1972; Hunt 1960) encountered during esocid stockings (Wahl and Stein 1989). Some walleye stockings occurred at lower water temperatures with associated longer evacuation rates; these effects could have resulted in overestimates of losses to predation for walleye (Santucci and Wahl 1993). As a result, estimates of differences between walleye and muskellunge may be conservative. However, the consumption rates of largemouth bass also decline with temperature (Rice and Cochran 1984). The combined effects of temperature on observed differences between species will be the result of complex interactions that require future evaluation.

System-specific characteristics such as the density and size distribution of potential predators, as well as the availability of alternative prey, may have influenced comparisons of predatory mortality among species. Previous studies found no relationships with predation mortality over the range of empty stomachs (40–60%) and largemouth bass densities (1–14/ha) observed for reservoirs examined in this study (Stein et al. 1981; Carline et al. 1986; Wahl and Stein 1989; Santucci and Wahl 1993). Several introductions fell outside of these narrow ranges for densities of largemouth bass capable of ingesting stocked fish: Bird's Pond for small esocids (75/ha) and Ridge Lake, 1991, for large walleye (0/ha). Deleting these two reservoir introductions does not substantially change the conclusions. In addition, introductions of small walleye in Ridge Lake experienced high effective largemouth bass densities (40–90/ha). These differences in densities serve to make estimates of differential vulnerability between species conservative. Although reservoir data suggest that walleye are less vulnerable to resident predators than muskellunge, only by simultaneously introducing similar sizes into the same reservoir can we make direct comparisons.

Fig. 3. Mean mortality attributable to largemouth bass predation for small (130–160 mm) and large (190–220 mm) sizes of walleye and esocids following introduction into Ohio and Illinois reservoirs. Values over bars are number of reservoir experiments; vertical lines are 95% confidence intervals.



I compared losses of introduced fish to largemouth bass because they are the dominant littoral predator in many lakes and reservoirs. In this study, few limnetic predator species existed that were large enough to prey on the sizes of fish stocked. However, given differences in habitat selection observed between walleye and muskellunge, other predators may be important in other bodies of water. Previous work and the current study suggest that to reduce losses to largemouth bass predation both species should be introduced in the fall at large sizes. My results suggest that largemouth bass predation is of less concern for introduction of walleye than for muskellunge across a range of sizes. However, walleye are often stocked at smaller sizes than those examined here (Ellison and Franzin 1992). There are few quantitative estimates of losses to predation for walleye less than 130 mm in length, except for the work of Santucci and Wahl (1993). Because prey size interacts with predator size and behavior to determine predation mortality, additional field evaluations are needed.

Understanding mechanisms influencing survival is critical to developing successful strategies for introducing fishes (Ellison and Franzin 1992). Predation can exert an important effect that will vary with behavior of stocked fish. Relative comparisons among other species regarding the importance of predation will allow us to generalize and develop predictions for other introduced fishes.

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Determining the most efficient method of age determination for estimating the age structure of a fish population[1]

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Abstract: Less precise, but economic methods for estimating the age of individual fish can provide better estimates of age structure than precise, but expensive methods. The benefits of using a precise ageing method can be compromised by its cost, which may restrict the size of the sample aged. If sample size is restricted, the effect of sampling error on an age structure may be greater than the effect of ageing error from a less precise ageing method that does not restrict sample size. We used Monte Carlo simulations to assess the relative size of sampling and ageing errors when estimating the age structure of populations of *Pomacentrus moluccensis* from the southern Great Barrier Reef, Australia. Sampling error associated with ageing less than 200 individuals was, on average, larger than the effects of most commonly reported ageing errors. Other factors that may complicate this comparison of ageing methods involve the financial cost of different methods and the logistics of sampling more fish.

Résumé : Les méthodes peu précises mais peu coûteuses d'évaluation de l'âge des poissons pris individuellement peuvent donner de meilleures estimations de la structure d'âge que les méthodes précises mais coûteuses. Les avantages d'une méthode précise peuvent être compromis par son coût, qui force à réduire la taille de l'échantillon étudié. Si la taille de l'échantillon est réduite, l'effet de l'erreur d'échantillonnage sur une structure d'âge peut être plus grand que l'effet de l'erreur sur l'âge produite par une méthode moins précise qui ne restreint pas la taille de l'échantillon. Nous avons employé des simulations de Monte Carlo pour évaluer le rapport entre la taille de l'échantillon et les erreurs sur l'âge dans l'estimation de la structure d'âge de populations de *Pomacentrus moluccensis* du récif sud de la Grande Barrière, en Australie. L'erreur due à l'échantillonnage associée à l'étude de moins de 200 spécimens était, en moyenne, plus grande que les effets des erreurs sur l'âge les plus couramment signalées. Les autres facteurs qui peuvent compliquer cette comparaison des méthodes de détermination de l'âge sont le coût financier des différentes méthodes et la logistique du prélèvement d'échantillons plus grands.
[Traduit par la Rédaction]

Introduction

A wide variety of techniques are used to estimate the age of fish (Boehlert 1985; Lai et al. 1987; Stevenson and Campana 1992). As well as differing in the time and cost required to determine the age of an individual fish, the techniques also exhibit a range of reliabilities (e.g., Lai et al. 1987). The most common techniques may not have significant biases (i.e., consistent deviation from the true value), but they do vary greatly in precision (i.e., variation among replicate estimates). In general, the frequency and magnitude of imprecise age determinations is inversely related to the time and cost of the technique. That is, more complicated and expensive techniques are generally not used unless they provide more precise estimates of age. Accurate and precise ageing techniques are most appropriate for the determination of growth rates and other demographic parameters that can be seriously biased by any ageing errors (Lai and Gunderson 1987; Bradford 1991).

An estimate of the age structure of a population is essential for many forms of stock assessment (see review by Megrey 1989). This is commonly derived by ageing a sample of individuals and calculating the relative abundance of each age-class. Unfortunately, while traditional methods for the determination of age in fish (such as annuli in sectioned otoliths) provide relatively precise estimates of age,

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they are often laborious and expensive, which can restrict the size of the sample that is aged. Perhaps as a consequence, there has been a great deal of research on methods to increase the efficiency of sampling designs to estimate age structure (e.g., Lai 1993). Similarly, a range of procedures has been developed to estimate age structure by ageing a subsample of individuals from a larger sample stratified by length (Kimura 1977; Hoenig and Heisey 1987; Kimura and Chikuni 1987). The common aim of these papers has been to increase the precision of an estimated age structure by reducing sampling error and keeping the cost (in time and money) of sampling fixed. A more simple solution to the problem of reducing the magnitude of sampling error is to increase the size of the sample of fish aged. For a fixed total cost, this can only be done by reducing the cost of determining the age of individual fish.

The weight of otoliths is known to be closely related to age in many fish species, and has been proposed as an economic and objective method of age determination (Boehlert 1985; Fletcher 1991; Worthington et al. 1995). Because this method is considerably more simple and inexpensive than traditional methods (e.g., Boehlert 1985), larger samples of fish can be aged for the same total cost. By ageing more individuals, and hence reducing sampling error, the use of otolith weight may result in better estimates of age structure. However, as with traditional methods of ageing, the use of otolith weight can lead to random errors and bias in the determination of age (Worthington et al. 1995). Such errors also affect the precision and accuracy of the estimated age structure. As a consequence, the potential reduction in sampling error by using otolith weight to age more individuals may be compromised by the effects of any increased ageing error.

In this paper, we use Monte Carlo simulations to investigate the relative effect of sampling error and ageing error on the precision of estimated age structures. These simulations were done to investigate the potential advantages of using otolith weight rather than annuli in sectioned otoliths. In practice, the results can be generalized to examine the compromise between sampling error and ageing error for any ageing method.

Methods

When an age structure is being determined, both sampling and ageing error can modify the estimated proportion of individuals within an age-class. The effects of sampling error can be assessed by calculating the variance in an estimated proportion at age ($\hat{p}_i$) by

$$\text{Var } \hat{p}_i = \frac{p_i(1-p_i)}{n}$$

where p_i is the true proportion of individuals in age-class i, and n is the total sample size.

To assess the effects of ageing error, the variance in an estimated proportion at age needs to be calculated differently. It is first necessary to construct a classification matrix that describes the relationship between true and observed ages (see also Richards et al. 1992). Most studies of ageing error report two main types of error: normally distributed imprecision and underageing bias (e.g., Kimura and Lyons 1991). Both these types of error can be incorporated into a classification matrix (**P**) with elements (p_{ij}) that represent the probability that a fish of age i will be estimated to be age j, using

$$p_{ij} = \frac{1}{\sigma_i\sqrt{2\pi}} \int_{j-0.5}^{j+0.5} \exp\left(\frac{-(j-\mu_i)}{2\sigma_i^2}\right) dA$$

where σ_i is the standard deviation (a measure of precision) and μ_i is the mean (a measure of bias) of estimated ages for individuals of age i, and A is the number of age-classes being considered. This was calculated for several values of normally distributed (CV = 0, 5, 10, 15, and 20%) and biased errors (bias = 100, 95, and 70%), chosen to represent the range commonly reported (e.g., Lai et al. 1987; Kimura and Lyons 1991; Worthington et al. 1995). The matrix (**P**) can then be used with the vector representing the true age structure (**T** with elements t_i) to estimate the vector of observed age structure (**O** with elements o_i),

$$\mathbf{O} = \mathbf{PT}$$

and the variance associated with an estimated proportion at age by

$$\text{Var } \hat{p}_i = (o_i - t_i)^2$$

The simultaneous effects of sampling and ageing errors cannot be calculated as simply, so the variance associated with an estimated proportion at age was calculated using Monte Carlo simulations. Sampling error was simulated by randomly selecting samples of 50, 100, 200, 500, 1000, and 2000 individuals from an age structure. Individual ages from these samples were then subjected to a range of ageing errors. These included both normally distributed and biased ageing errors of the ranges described above. The estimated ages with error (Y) were calculated using

$$Y = bX + N(0,cX)$$

where X is the true age sampled, b is a measure of bias, and c is a measure of ageing error corresponding to the coefficient of variation described by Chang (1982). Estimated ages with error were then converted to proportions and their variance calculated using

$$\text{Var } \hat{p}_i = (\hat{p}_i - p_i)^2$$

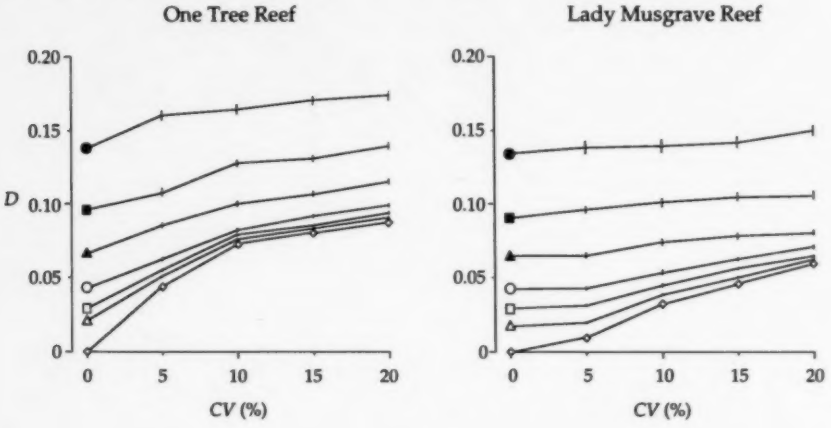
This was repeated 100 times for each combination of sampling and ageing error.

Although the effects of sampling and ageing errors on an estimated proportion at age can be assessed by the variance of the estimate, the effects of sampling and ageing errors on an age structure need to be calculated differently. An index of the effects of sampling and ageing errors on an age structure was calculated using the variances in estimated proportions at age for all A age-classes by

$$D = \sqrt{\sum_{i=1}^{A} \text{Var } \hat{p}_i}$$

Consequently, the index D can be considered a measure of average Euclidean distance between the true and estimated age structures in an A-dimensional space (see also Lai and Gundersen 1987) and D^2 corresponds to the vartot of

Fig. 1. Mean and standard error (vertical lines) of D (an index of error in an age structure) for a range of sampling and unbiased ageing errors on two reefs. Points determined exactly are shown by symbols; others were determined by Monte Carlo simulations. Lines join points with equal sample size. ●, 50 individuals; ■, 100 individuals; ▲, 200 individuals; ○, 500 individuals; □, 1000 individuals; △, 2000 individuals; ◇, ∞. Sampling errors are independent of ageing error when CV = 0, and ageing errors are independent of sampling error when $n = \infty$.



Kimura (1977). This index was calculated from the variances in estimated proportions at age caused by sampling error, ageing error, and both in combination.

The effects of sampling and ageing errors on estimates of age structure are dependent on the shape of the age structure itself (e.g., different longevities or frequency of dominant age-classes). For example, age structures with more old fish will generally be exposed to larger and more frequent ageing errors. All simulations were based on the age structure of *Pomacentrus moluccensis* sampled from One Tree Reef and Lady Musgrave Reef in the Capricorn–Bunker region of the southern Great Barrier Reef, Australia. These reefs were chosen to represent the range in age structures sampled from seven reefs in the region (see Doherty and Fowler 1994). The age structure from Lady Musgrave Reef contains a low proportion of old individuals with no outstanding year-classes. In comparison, the age structure from One Tree Reef contains a large proportion of old individuals and has several strong year-classes. Results may differ if age structures of considerably different shape are used.

Results

The independent effects of sampling error and ageing error

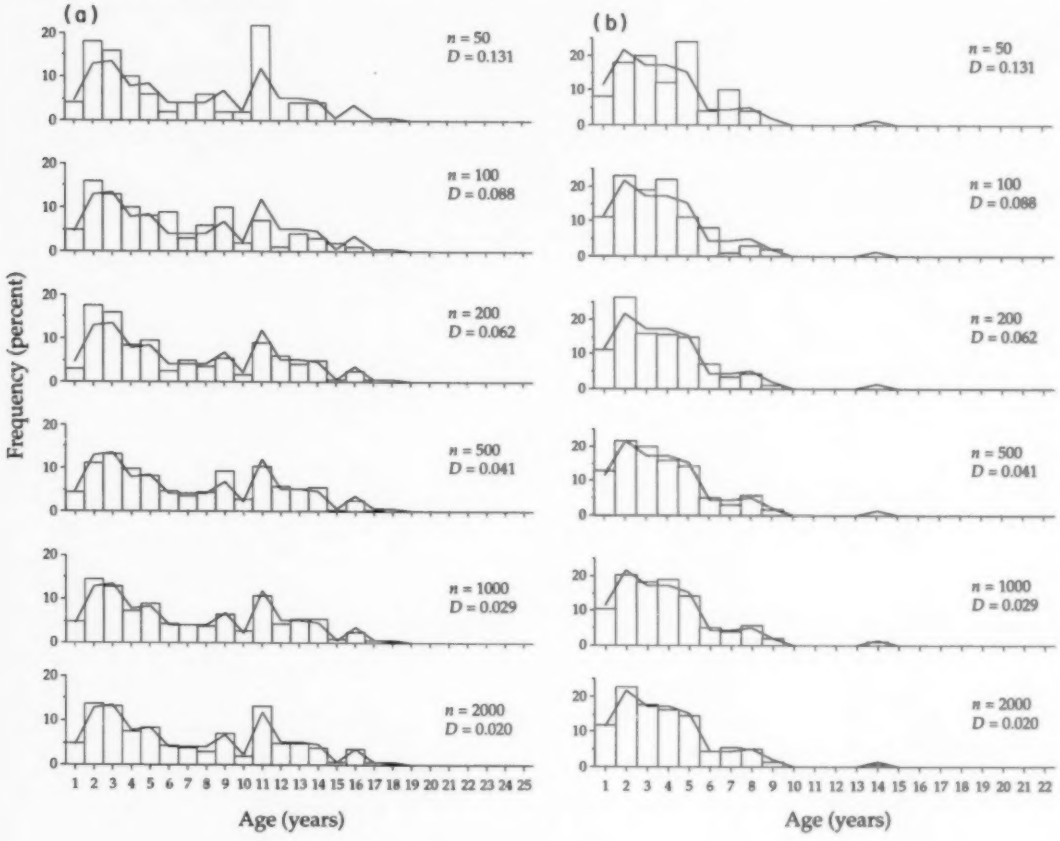
In the absence of any ageing error, estimates of age structure improved as sample size increased. The magnitude of improvement in the estimate of age structure decreased as sample size increased (Fig. 1). For example, samples of 50 or 100 individuals provided poor estimates of the true age structure on both reefs (Fig. 2). Because of the random nature of sampling error, strong year-classes were often made to look weak, or weak year-classes made to look strong. By increasing the number of individuals sampled to 500, the precision of the estimated age structure was greatly increased, but there was a comparatively smaller increase in precision when sample size was further increased to 1000 (Figs. 1 and 2).

In the absence of any sampling error, estimates of age structure improved as ageing error decreased (Fig. 1). This was particularly apparent for the age structure from One Tree Reef, which contained several outstanding year-classes (Fig. 3*a*). For example, the strength of the 11th year-class on One Tree Reef was increasingly underestimated as ageing errors became larger. Similarly, the strength of the weak 10th year-class on One Tree Reef was increasingly overestimated as ageing error became larger. In contrast, there were no outstanding year-classes in the age structure from Lady Musgrave Reef (Fig. 3*b*), and ageing error had a smaller effect (Fig. 1). The effects of ageing errors on both age structures were largely predictable, with increasing error reducing the differences in strength among year-classes (Fig. 3).

The interaction between sampling error and ageing error

The effects of ageing error on estimates of age structure increased with sample size. For example, the difference in precision between age structures estimated by 1000 and 2000 individuals decreased as ageing error increased (Fig. 1). As a consequence, the advantages of increasing sample size, and hence reducing sampling error, were reduced as ageing error increased. In general, the effects of increasing ageing error were again predictable, with increased error reducing the difference in strength among year-classes (e.g., Fig. 3). That is, the abundance of strong year-classes was consistently underestimated and that of weak year-classes overestimated. Increasing ageing error

Fig. 2. True (line) and examples of simulated (bars) age structures from (*a*) One Tree Reef and (*b*) Lady Musgrave Reef with a range of sampling errors (i.e., *n*) in the absence of ageing error. The examples shown represent the age structures with the median value of *D* for the 100 simulations. That is, half the simulated age structures were less similar to the true age structure than those shown.

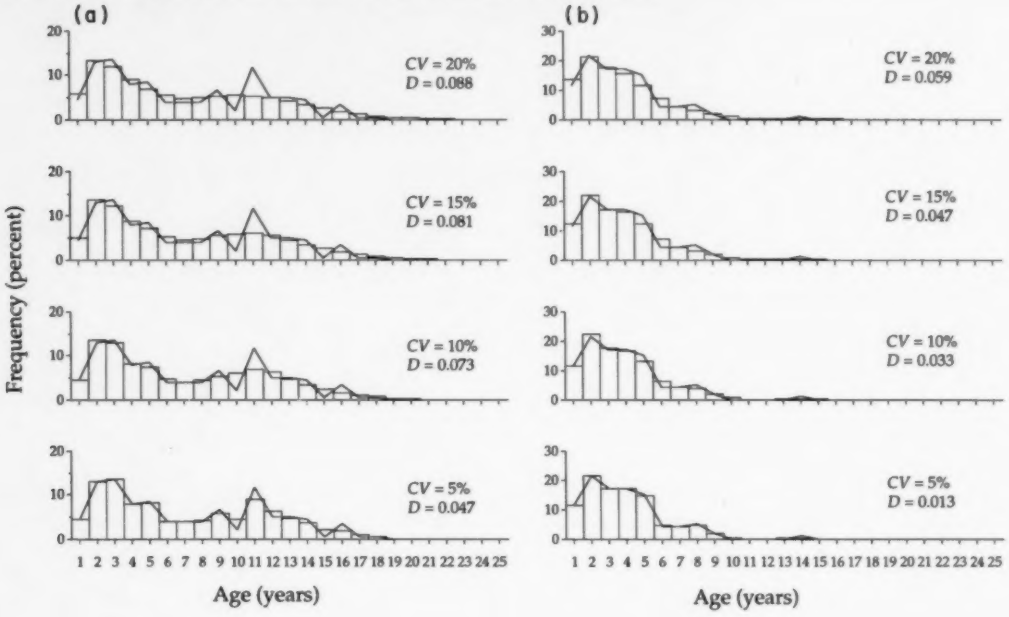


generally affected estimates of age structure from Lady Musgrave Reef less than estimates of age structure from One Tree Reef (Fig. 1).

Estimates of error in ageing *P. moluccensis* using either annuli in sectioned otoliths or otolith weight vary among reefs (CVs ranging from 5–16%), although, on average, annuli provide more reliable estimates of age (Fowler and Doherty 1992; Worthington et al. 1995). In contrast, the processing time to determine an age using otolith weight is approximately 5–10 times shorter than when using annuli, in theory allowing 5–10 times more fish to be aged by otolith weight. Assuming a CV of ageing error of 5% for the use of annuli and 10% for the use of otolith weight, we can determine which will result in a better estimate of age structure. If only 100 fish could be aged by annuli or 500 by otolith weight, the use of otolith weight would on average provide a more precise estimate of the true age structure on both reefs (Fig. 1). Even if 200 fish could be aged by annuli, otolith weight would still provide a more precise estimate of the true age structure on the basis of a sample of 500 individuals. In contrast, if 500 fish could be aged by annuli they would provide a more precise estimate of the age structure on each reef than 2000 fish aged by otolith weight. At low sample sizes (50–200), sampling error is much larger than ageing error, but as sample size increases (500–2000), sampling error decreases and ageing error dominates.

When the ageing method is biased, more precise estimates of age structure will often be gained from ageing methods with normally distributed ageing error (Fig. 4). For large amounts of bias, better estimates of age structure will come from highly imprecise methods. For example, if 100 fish were sampled from One Tree Reef and aged using a method that provided slightly biased (b = 0.95) estimates of age, better estimates of age structure would be provided by methods that were also imprecise (CV = 5–10%; Fig. 4*a*). By using a more biased ageing method (b = 0.70), better estimates of age structure would be provided by very imprecise methods of age determination (CV = 20%; Fig. 4*b*).

Fig. 3. True (line) and simulated (bars) age structures from (*a*) One Tree Reef and (*b*) Lady Musgrave Reef with a range of ageing errors (i.e., CV) in the absence of sampling error.



Discussion

Comparing sampling and ageing error

Errors in the ageing of individuals, of the same magntiude as commonly reported (i.e., CV = 5–15%; Kimura and Lyons 1991), modified estimates of age structure. For a given sample size, estimates of age structure were most reliable when there were small ageing errors. An ideal method to estimate age structure would sample large numbers of fish and age them with little error. Unfortunately, accurate and precise ageing methods are often costly (in time and money), which can logistically restrict the number of fish aged in a sample. When logistic constraints reduce the size of a sample, sampling error will increase and modify estimates of age structure. As a result, the benefits of using a reliable ageing method can be compromised by an increase in sampling error. Provided estimates of both potential ageing errors and logistic constraints on sample size are known, the ageing method that provides the most reliable estimate of age structure can be chosen using simulations similar to those described here.

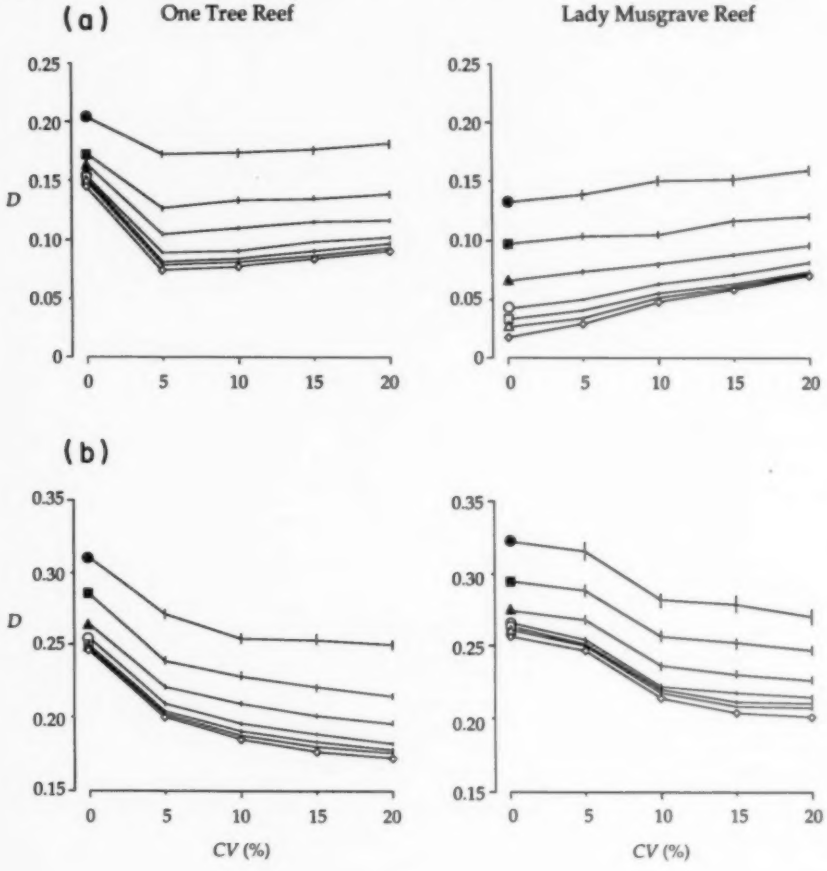
Whilst both ageing and sampling error affected estimates of age structure, each modified the true proportion of individuals in an age-class in different ways. As the effects of sampling error were generated by a random process, they were unpredictable, with strong year-classes often made weak or weak year-classes made strong. In contrast, the effects of ageing errors on the estimated age structure were largely predictable. Normally distributed ageing errors reduced the differences among the strength of year-classes (i.e., smoothed the age structure), whilst biased errors shifted or compressed the age structure. These errors can bias the subsequent use of age structures to estimate some parameters (Barlow 1984), but because the effects of ageing error are predictable, they can be corrected for (see Richards et al. 1992), unlike those caused by sampling error.

There are several factors that have the potential to complicate the general comparison of ageing and sampling errors among ageing methods. In particular, the logistics of sampling will determine how many individuals can be sampled, and hence the magnitude of sampling error. For example, the sample size may be restricted by the time taken to collect individuals or by the time taken to remove and prepare their otoliths. Variation in the error caused by an ageing method may also complicate any general comparison of ageing methods. Spatial variation in the clarity of annuli in otoliths has the potential to modify the reliability of ageing and also increases the processing time required for an individual age determination (Fowler and Doherty 1992). As a result, what may be an efficient and reliable technique in some areas may be laborious and error prone in others.

Potential of otolith weight to estimate age structure

Otolith weight can be used to estimate the age of individuals from many fish species (e.g., Boehlert 1985). As with other ageing methods, the use of otolith weight can result in imprecise and biased estimates of age (Worthington et al. 1995). For species that can be aged reliably from annuli within their otoliths, the use of otolith weight will, in general, result in more ageing errors than the use of annuli. This will occur as annuli within otoliths are often used to establish the relationship between otolith weight

Fig. 4. Mean and standard error of D for a range of sampling errors with (*a*) slightly biased ($b = 0.95$) and (*b*) heavily biased ($b = 0.70$) ageing errors on two reefs. See caption of Fig. 1 for further details.



and age. As a result, otolith weight can only provide estimates of age as reliable as those on which the initial calibration was done. In contrast, because of the efficiency of age determination using otolith weight, it provides the opportunity to age more fish and hence to reduce sampling error.

Because of the potential for variation in the relationship between otolith weight and age, the relationship needs to be recalibrated for each new sample of fish. To maximize the potential benefits of using otolith weight, recalibration requires accurate and precise estimates of age from a relatively small number of fish (see Worthington et al. 1995) by techniques such as the use of annuli within otoliths. Particular attention should be paid to the interpretation of annuli within these otoliths so that estimates of age are as accurate and precise as possible. These estimates of age can also be used to estimate other parameters, such as growth rates, which can be severely affected by ageing errors (Lai and Gunderson 1987). A more economic method of age determination, such as the use of otolith weight, can then be used to age the large number of fish required to reduce the effects of sampling error on an estimated age structure. Individual age determinations may be less precise, but if these errors can be quantified, their effects on an age structure can be estimated and corrected (Richards et al. 1992).

The potential of using more than one ageing method to improve estimates of age structure appears largely to have been overlooked. As the precision of most ageing methods varies with age, the method that provides the fewest errors (i.e., from both ageing error and sampling error) may also differ among age groups. Consequently, techniques such as otolith weight, which can provide quick and reliable estimates of the age of young fish, are likely to be the most efficient to age the large numbers of young fish required for an age structure. Alternatively, the smaller numbers of old fish will probably be most efficiently aged by more reliable methods such as annuli within sectioned otoliths. By quantifying both the time taken and potential errors of a range of ageing methods, their relative efficiency can be calculated for each age group in an age structure. The combination of ageing methods that provides

the most efficient and precise estimate of age structure can then be calculated.

A wide variety of techniques can be used to estimate the age of fish. Once age can be determined reliably, it provides estimates of parameters such as growth, mortality, and age structure that can be used to enhance management of a fishery. While more reliable methods of age determination are preferable for estimation of some parameters (e.g., growth), less reliable techniques that are also economic may be preferable when large numbers of individual age determinations are needed (e.g., in the estimation of age structure by otolith weight or hatch date distribution by methods such as those of Ralston and Williams 1988). By suggesting this, we are not simply recommending the use of inaccurate or imprecise techniques of age determination. Instead, we are recommending their use only when other types of error, which may have larger effects on estimates of age structure, can be reduced.

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Regeneration of nitrogen and phosphorus by bluegill and gizzard shad: effect of feeding history

Martha E. Mather, Michael J. Vanni, Thomas E. Wissing, Scott A. Davis, and Maynard H. Schaus

Abstract: We combined laboratory and field studies to experimentally assess how the effects of feeding regime and time since feeding influence nitrogen (N), phosphorus (P), and the N:P ratio excreted by two common freshwater fish, bluegill (*Lepomis macrochirus*) and gizzard shad (*Dorosoma cepedianum*). In addition, for adult gizzard shad, we modelled excretion rates as a function of the nutrient content of ingested sediment detritus. For both bluegill and gizzard shad, feeding significantly increased nutrient excretion rates and altered excreted N:P ratios. For both species, excretion rates were highest immediately after feeding and declined thereafter. Because the phosphorus excretion rate decreased more rapidly after feeding than did the nitrogen excretion rate, the excreted N:P ratio increased with time since feeding. Young-of-year gizzard shad excreted more nitrogen than adults, resulting in a higher excreted N:P ratio for these small fish. For P, predictions from our model agreed well with our experiments with gizzard shad; for N, the agreement was not as strong yet was still reasonable. In summary, N:P ratios excreted by these fish differed across species, size, and time since feeding. Variation in these factors may explain discrepancies among studies that examine both trophic interactions and nutrient budgets.

Résumé : Nous avons combiné les études de laboratoire et les travaux de terrain pour évaluer expérimentalement les effets du mode d'alimentation et du temps écoulé depuis la prise d'aliments sur l'excrétion de l'azote (N) et du phosphore (P) et sur le rapport N:P à l'excrétion chez deux poissons dulcicoles communs, le crapet arlequin (*Lepomis macrochirus*) et l'alose à gésier (*Dorosoma cepedianum*). De plus, chez les aloses à gésier adultes, nous avons modélisé les taux d'excrétion en fonction du contenu en nutriments des sédiments ingérés. Tant chez le crapet arlequin que chez l'alose à gésier, l'alimentation faisait augmenter de façon notable le taux d'excrétion des nutriments et modifiait le rapport N:P à l'excrétion. Chez les deux espèces, les taux d'excrétion étaient au plus haut juste après la prise de nourriture, et baissaient par la suite. Étant donné que le taux d'excrétion du phosphore baissait plus rapidement après l'ingestion que celui de l'azote, le rapport N:P à l'excrétion augmentait avec le temps écoulé depuis la prise de nourriture. Chez l'alose à gésier, les jeunes de l'année excrétaient plus d'azote que les adultes, de sorte que leur rapport N:P était plus élevé que celui des poissons de plus grande taille. En ce qui concerne P, les prédictions de notre modèle concordaient bien avec les expériences faites sur l'alose à gésier; en ce qui concerne N, la concordance était moins forte, mais restait acceptable. Pour résumer, les rapports N:P à l'excrétion différaient selon les espèces, la taille des poissons et le temps écoulé depuis la prise de nourriture. Les variations de ces facteurs peuvent expliquer des discordances entre les résultats d'études qui examinent à la fois les interactions trophiques et les bilans des nutriments.

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Introduction

A number of studies have examined the response of organisms in multiple trophic levels to predator or nutrient alterations (Carpenter et al. 1985; McQueen et al. 1986; Carpenter and Kitchell 1988; McQueen and Post 1988; Power 1990). The results of these manipulations often vary across systems, trophic levels, and time. Because of this variation, the relative importance of top-down and bottom-up effects has been debated (DeMelo et al. 1992; Carpenter and Kitchell 1992; Matson and Hunter 1992; Hunter and Price 1992;

Power 1992; Strong 1992; Menge 1992). The strength of direct linkages between key functional groups at adjacent trophic levels can affect the importance of top-down effects (Paine 1980). Because nutrient regeneration by animals is a potentially important indirect effect that can reduce the strength of direct trophic linkages via feedback loops, knowledge of how consumers in lakes (e.g., fish and zooplankton) recycle nitrogen and phosphorus can provide insight into reasons for across-system variation in response to trophic perturbations (Caraco 1993; Carpenter et al. 1992).

Because phosphorus and at times nitrogen are limiting nutrients for freshwater phytoplankton (Schindler 1977; Elser et al. 1990), these nutrients can influence both algal biomass and community structure (Tilman et al. 1982). Thus, it is important to understand how various consumers recycle and transport nutrients in aquatic food webs (Sterner 1990; Caraco 1993; Vanni 1995). Nutrient excretion by herbivorous zooplankton has long been recognized as an important source of nutrients to phytoplankton (Goldman et al. 1979; Lehman 1980; Lehman and Sandgren 1985). However, the importance of nutrient excretion by fish has been debated. Some investigators have found that nutrient excretion by fish is an important source of phosphorus (Lamarra 1975; Brabrand et al. 1990; Kraft 1992; Schindler et al. 1993), increases primary productivity (Lamarra 1975; Brabrand et al. 1990; Schindler et al. 1993), and alters phytoplankton species composition (Brabrand et al. 1990; Vanni and Findlay 1990). Others have concluded, however, that the absolute amount of phosphorus regenerated by fish is unimportant relative to other sources (Kitchell et al. 1975; Nakashima and Leggett 1980). Findings that fish diet (Lamarra 1975; Brabrand et al. 1990), size (Lamarra 1975; Brabrand et al. 1990; Kraft 1992; Schindler et al. 1993), diel movements (Brabrand et al. 1990; Carpenter et al. 1992*a*; Schindler et al. 1993), and community composition (Schindler et al. 1993) alter the importance of fish nutrient regeneration by altering rates, stoichiometric ratios, and the spatial–temporal distributions at which nutrients are excreted further complicate system-wide effects of nutrient excretion by fish. The effects of fish size and ration on nutrient excretion are well known under laboratory and aquaculture conditions (e.g., Weisberg and Lotrich 1982; Cui and Wooten 1988; Weissman et al. 1988). However, it is not clear how these factors affect nutrient recycling by fish fed natural prey. Because these ecological and behavioral considerations vary across systems, understanding how these factors influence nutrient excretion by fish could explain some of the ambiguity regarding the importance of excretion by fish.

Models are frequently used to examine nutrient recycling by fish (Nakashima and Leggett 1980; Kraft 1992; Schindler et al. 1993; Vanni 1995) and are essential tools for understanding whole-system function. However, we need experimental data to accurately parameterize these models. In addition, how fish affect either the amount or distribution of nutrients rarely has been directly quantified, nor have the effects of feeding regime, especially time since feeding, been examined under natural food conditions. Furthermore, simultaneous measurements of the nitrogen and phosphorus excretion rates of fish are rare. Herein, we combine laboratory and field studies to experimentally assess the effects of feeding on the rates and ratios at which nitrogen and phosphorus are excreted by two common freshwater fish, bluegill (*Lepomis macrochirus*) and gizzard shad (*Dorosoma cepedianum*). Specifically, we quantify nutrient excretion by (*i*) bluegills fed chironomid larvae in the laboratory, (*ii*) laboratory-maintained and field-collected adult gizzard shad, and (*iii*) field-collected, young-of-year gizzard shad. (*iv*) In addition, using a simple model that predicts excretion of gizzard shad as a function of sediment nutrient content, we compare predicted and measured rates.

Methods

Bluegills in the laboratory

To assess the effects of feeding and time since feeding on nutrient excretion by bluegill, in May 1991 we quantified nitrogen and phosphorus excretion rates in the following three laboratory treatments: (*i*) unfed bluegill ($N = 4$), (*ii*) bluegill fed chironomid larvae ($N = 4$), and (*iii*) water only (which controlled for changes in nutrient concentrations that may occur independent of fish; $N = 5$). Bluegill, with a mean total length (TL) of 50.6 mm and a mean weight of 1.96 g wet mass, were obtained from Osage Catfisheries (Osage Beach, MO 65065, U.S.A.) in April 1991 and acclimated to the laboratory for at least 2 weeks prior to experiments. Like all experimental fish, these bluegill were acclimated to the laboratory at 18–22°C and fed an ad libitum diet of crushed Purina trout chow. Treatments were randomly assigned to 20-L test tanks containing one-third dechlorinated tap water, two-thirds deionized water, no substrate, and one airstone. Tanks were housed in an environmental chamber maintained at 20°C with a 14 h light : 10 h dark photoperiod. In the communal acclimation tank, prior to experiments, all fish were fed to satiation and then starved for 12 h. Ten fish were then transferred into each test tank (mean biomass per tank = 19.58 g; mean tank density = 0.98 $g \cdot L^{-1}$) and starved for an additional 24 h for a total pre-experimental starvation time of 36 h. At the end of this acclimation period, half the water in each test tank was replaced with deionized water to reduce pre-experimental levels of nitrogen and phosphorus. This protocol reduced background levels of nutrients yet allowed bluegill to recover from the handling stress associated with tank transfer. Each bluegill in the fed treatment was given a total of 30 laboratory-cultured chironomid larvae (*Chironomus riparius*; 30 larvae = 0.15 g total ration per fish or 7.8% of fish wet body mass). To maximize consumption, bluegill were fed 20 chironomids initially, at time 0 h, followed by an additional 10 larvae per fish, 4 h later. Chironomids were individually dropped into tanks to ensure that all fish had equal access to this food. All prey were consumed immediately. The experiment officially began when the initial feeding was completed (time 0 h).

Samples were taken from each test tank at the following times: (*i*) before fish were added to ensure that initial conditions were similar (data not shown), (*ii*) at the end of the 24-h test tank acclimation (experimental time 0 h), and at (*iii*) 4 h, (*iv*) 8 h, and (*v*) 24 h after initial feeding was completed. Test water was collected with an acid-washed,

Plexiglas tube sampler. Water was filtered immediately through a Gelman A/E glass fiber filter, stored in acid-washed Nalgene bottles, and refrigerated. Ammonia-N (N) and soluble reactive phosphorus (P) were analyzed using the Solorzano colorimetric and acid molybdate techniques, respectively, within 24–48 h of collection (Wetzel and Likens 1990).

Adult gizzard shad in the laboratory

Adult gizzard shad (mean TL = 150 mm, mean weight = 18.7 g) were collected by electroshocking from Acton Lake, Butler, and Preble counties, Ohio, in April 1991 and acclimated to the laboratory for at least 4 weeks prior to experiments. In May 1991, we carried out the following three treatments in the laboratory: (*i*) fish were exposed to sediments but prevented from feeding ($N = 3$), (*ii*) fish were allowed to consume sediments ($N = 3$), and (*iii*) water only ($N = 3$). About 2 cm of fresh sediments, collected from the top 1 cm of the Acton Lake littoral zone within 2 weeks of the experiments, was present in each tank containing fish. The temperature, water, light regime, acclimation procedures, and water chemistry protocols were identical to those described for bluegill. To begin the experimental acclimation period, we transferred five fish into each 65-L test tank (mean biomass per tank = 93.53 g; mean tank density = 1.43 $g \cdot L^{-1}$). Fish were prevented from feeding for an additional 24 h by screens composed of a rigid Plexiglas frame surrounding a cotton net interior, which separated them from the sediments but still allowed nutrient exchange to take place during the acclimation period. Then, one half of the water in each test tank was replaced with deionized water, and in the fed fish treatment, bottom screens were removed and fish were allowed to feed for 24 h (experimental time 0 h). Samples were taken from each tank at the following times: (*i*) before fish were added (data not shown), (*ii*) at the end of the 24-h test tank acclimation, i.e., time 0 h, (*iii*) at 8 h, and (*iv*) at 24 h after the removal of the screens. Test water was collected from each tank with an acid-washed, 1.25 cm diameter Tygon tubing siphon permanently installed in the center of the tank. In each of two additional tanks, 10 fish were allowed to feed throughout the experiment. We examined the gut contents of these fish and although they appeared to behave normally in the test tanks, the fish consumed little sediment. Consequently, and because excretion rates were similar between treatments, we grouped all of these laboratory-tested gizzard shad together into a single unfed fish treatment ($N = 6$).

Adult gizzard shad in the field

To quantify the N and P excretion rates of actively feeding fish, field experiments were conducted with gizzard shad collected from Acton Lake on 21 May 1992 (surface temperature 18.5°C), and 10 June 1992 (surface temperature 21°C). As no practical way existed to measure the non-feeding excretion rates of these field-collected fish, in these experiments only two treatments were used: (*i*) fish that had fed ($N = 5$), and (*ii*) water only ($N = 5$). Data from the water-only treatment are not shown because nutrient concentrations did not change significantly. Gizzard shad were collected by electroshocking in late morning because previous studies have shown that in Acton Lake, gizzard shad guts become full by that time (Pierce et al. 1981). Both observation of guts from fish collected at the same time as these experimental fish and subsequent fecal production by these experimental fish indicated that our field-collected gizzard shad, in fact, were eating sediments even though rations of individual fish were uncontrolled and likely varied. In the fish treatment, one gizzard shad was placed into each 12-L test tank filled with deionized water (first set of experiments, May 1992, mean weight 23.27 g; second set of experiments, June 1992, mean weight 48 g), resulting in mean densities of 1.96 and 4.0 $g \cdot L^{-1}$ for the first and second experiments, respectively. In both sets of field experiments, samples were taken before the fish were placed in tanks (0 h), and at 1, 4, 8, and 24 h after fish were collected. All other methodological details were the same for both sets of experiments, and in the subsequent discussion these treatments are referred to as the fed fish treatments (Fed-1, Fed-2).

Young-of-year gizzard shad in the field

Young-of-year gizzard shad (mean TL = 53 mm, wet weight = 2.2 g; $N = 4$) were collected by electroshocking from Acton Lake on 29 July 1993 (surface temperature 28°C) to examine the N and P excretion rates of these young fish. These fish were collected in late morning (approximately 10:00) and thus we assume that they were feeding at the time of collection (Pierce et al. 1981). Individual fish were placed in 1.25-L Nalgene containers (fish density 1.8 $g \cdot L^{-1}$) containing Acton Lake water that had been previously filtered through a 0.7-μm glass fiber filter to remove algae and bacteria. Samples were taken before fish were added and at 1, 2, 4, and 8 h after capture. Water chemistry procedures were the same as described above.

Test for electroshocker effects on excretion rates

Our method of collecting gizzard shad, electroshocking, could have affected nutrient excretion rates and thereby biased our results. Therefore, we conducted an experiment to assess the effects of electroshocking on gizzard shad excretion rates. During acclimation, 16 young-of-year gizzard shad, maintained in the laboratory from October 1993 until February 1994, were divided between two 80-L, flow-through tanks housed in an environmental chamber, maintained at 12.5°C. Eight control fish were removed and placed in individual, 4-L acid-washed polyethylene containers. The remaining eight fish were stunned with a pulsed-DC backpack electroshocker, netted, and placed individually in 4-L containers identical to those used for control fish. The handling time for stunned fish was more than double that for control fish, and was similar to that of field-collected adult fish used in previous experiments. Electroshocked fish appeared to recover within 2 min and always behaved normally after 5 min. Samples, taken immediately after fish transfer (0 h) and at 1 h post-transfer, were analyzed as described above.

Analyses

Initial N and P concentrations (i.e., at experimental time 0 h) varied among experiments. Therefore, to standardize excretion among experiments and show patterns, cumulative

gross N and P excretion (total amounts of N and P excreted) was calculated as the change in N and P from the beginning of the experiment through 24 h. Cumulative gross excretion was then divided by fish biomass (wet mass) and time to obtain excretion rates, expressed as micrograms ammonia-N or soluble reactive P excreted per gram wet mass each hour so that all data could be compared regardless of fish biomass or tank size. Values in micrograms were converted to molar units by dividing by molecular weight (N, 14; P, 31). Both units are shown below.

For adults of both species, a two-way analysis of variance (ANOVA) was then used to examine how feeding regime and time since feeding influenced the excretion rates of N and P and the N:P ratio excreted. For young-of-year gizzard shad, a one-way ANOVA tested the effect of time. We also compared the excretion rates and ratios of young-of-year gizzard shad with those of field-collected adult gizzard shad using a two-way ANOVA with treatment (young-of-year, Fed-1, Fed-2) and time interval (0–1, 1–4, 4–8 h) as factors. When necessary, data were transformed (log X or log(X + 1)) to stabilize the variance. Tukey multiple comparisons were used to test for within-experiment, between-treatment differences.

Model

Recent studies have attempted to estimate excretion rates of fish by using bioenergetics models (Kraft 1992; Schindler et al. 1993). Using this method, nutrient assimilation rates (amount of N or P assimilated across the gut wall) are estimated by multiplying consumption rates (obtained from bioenergetics models) by assimilation efficiencies (percentage of consumed N or P that is assimilated). Then the rates at which nutrients are allocated to growth (obtained from fish growth rates and the nutrient content of fish tissues) are subtracted from assimilation rates to estimate excretion rates. We think it is useful to compare our measured excretion rates with those predicted by models but we chose not to use the above-described bioenergetics approach to estimate consumption. Instead, we modified a general mass balance model (Vanni 1995) for predicting excretion rates as a function of nutrient content and sediment detritus consumption.

In our modified model, following Mundahl (1991), we estimated consumption of sediment detritus (dry mass consumed per fish per day) using empirical relationships between consumption rates and temperature, fish wet mass, and hours of daylight (Salvatore et al. 1987; Vanni 1995). Using an average fish wet mass (23.3 g (Fed-1) and 48.0 g (Fed-2)) and Acton Lake temperatures, and assuming 14.5 and 15.0 h of daylight, respectively, we estimated consumption rates of 1.82 and 4.44 g dry mass sediment detritus·fish^{-1}·day^{-1} for the model outputs on the basis of Fed-1 and Fed-2 data. Nutrient (N and P) consumption rates can then be estimated if the nutrient contents of ingested detritus are known. We used Mundahl and Wissing's (1988) estimate of 0.021 g N·g dry mass^{-1} for sediment detritus consumed by Acton Lake gizzard shad in our calculations. Although Mundahl and Wissing (1988) did not quantify the P content of Acton Lake detritus, data collected in 1993 showed that sediment detritus ingested by gizzard shad contained 0.0041 g P·g dry mass^{-1} (average of eight fish; M.H. Schaus and M.J. Vanni, unpublished data) and we used this value.

We multiplied dry mass consumption (grams sediment detritus per day) by the nutrient content of ingested detritus (grams N or P per gram sediment) to estimate nutrient consumption rates of gizzard shad (grams N or P consumed per fish per day). Nutrient consumption rates were multiplied by assimilation efficiencies of 80% for N (average of values reported in Table 1 of Mundahl and Wissing (1988)) and 68% for P (M.H. Schaus and M.J. Vanni, unpublished data) to obtain nutrient assimilation rates (N or P assimilated per fish per day). To estimate N and P excretion rates (N or P excreted per fish per day), we subtracted nutrients allocated to growth from assimilation rates. This growth rate, estimated as 0.127 g wet mass·fish^{-1}·day^{-1}), was obtained from the average daily growth rates for age-1 Acton Lake gizzard shad reported by Pierce (1977). We converted this to nutrient allocation rates (N or P incorporated into biomass per fish per day) by assuming a dry mass to wet mass ratio of 0.2, a body N content of 0.08 g N·g dry mass^{-1}, and a body P content of 0.029 g P·g dry mass^{-1} (M.H. Schaus and M.J. Vanni, unpublished data). We converted nutrient excretion rates to units of micromoles N or P per gram wet mass per day to correspond to the units used in our experiments. To compare our experimentally measured excretion rates with model output, we converted the hourly rates we present in Fig. 1 to daily rates by averaging the mean hourly excretion rates of actively feeding fish (0- to 1-h intervals) and nonfeeding fish (average of 1- to 4-, 4- to 8-, and 8- to 24-h intervals) weighted by the hours of feeding (daylight) and nonfeeding (darkness).

Results

Excretion rates

For both species, the cumulative levels of N and P were higher in tanks with fish relative to those with water alone. When no fish were present, the N and P concentrations did not change through time, but with fish, the concentrations of both nutrients increased as the experiment progressed (data not shown). Because rates, rather than cumulative concentrations, are the variables of interest to nutrient budgets, below we examine the excretion rates of N and P and the excreted N:P ratio. For each species–nutrient combination, we examined the effects of feeding regime (fed vs. unfed), time since feeding, and, when relevant, the interaction between feeding regime and time scale. Throughout the remainder of the Results section, excretion rates are given as micromoles N or P excreted per gram fish wet mass per hour with values in micrograms per gram per hour given in parentheses for comparison with other studies.

Nitrogen excretion rates for bluegill

Mean N excretion rates ranged from 0.9 to 1.1 μmol·g^{-1}·h^{-1} for unfed bluegill (12.0–15.6 μg·g^{-1}·h^{-1}), and from 2.5 to 3.4 μmol·g^{-1}·h^{-1} for fed bluegill (34.5–47.0 μg·g^{-1}·h^{-1}; Fig. 1A). Fed fish excreted more N than unfed fish at all times (Fig. 1A; feeding effect, $P \leq 0.01$ (Tukey $P \leq 0.0001$); Table 1). Overall, N excreted by fed fish decreased through

Table 1. Results of ANOVAs that test the effects of feeding and (or) time since feeding on excretion rates of ammonia-N (μmol N·g fish^{-1}·h^{-1}) and soluble reactive phosphorus (μmol P·g fish^{-1}·h^{-1}), and the ratio of excreted N:P for bluegill and gizzard shad.

	N excretion rate	P excretion rate	N:P ratio
Bluegill			
P value			
Feeding (fed, unfed)	<0.01****	<0.01***	<0.01**
Time (0–4, 4–8, 8–24 h)	<0.01**	<0.01***	<0.01**
Interaction	0.94	<0.01**	0.12
Degrees of freedom			
Model	5	5	5
Error	18	18	18
Adult gizzard shad (all time intervals)			
P value			
Treatment (Fed-1, Fed-2, unfed)	<0.01**	<0.01****	<0.01****
Time (0–8, 8–24 h)	0.06	<0.01**	<0.01***
Interaction	<0.02*	0.03*	<0.01**
Degrees of freedom			
Model	5	5	5
Error	26	26	25
Adult gizzard shad (early time intervals only)			
P value			
Treatment (Fed-1, Fed-2)	0.03*	<0.01**	<0.01***
Time (0–1, 1–4, 4–8 h)	<0.01****	<0.01****	0.06
Interaction	0.77	0.26	0.47
Degrees of freedom			
Model	5	5	5
Error	24	24	22
Young-of-year gizzard shad			
Time (0–1, 1–2, 2–4, 4–8 h)	0.71	<0.01****	<0.03*
Degrees of freedom			
Model	3	3	
Error	12	12	12

Note: Adult gizzard shad data were analyzed in two ways, one using all time intervals and the other using only early time intervals. Bad samples occasionally produced unequal degrees of freedom for N, P, and N:P analyses within an experiment.

*$P \leq 0.05$ (Tukey's multiple comparison).

**$P \leq 0.01$ (Tukey's multiple comparison).

***$P \leq 0.001$ (Tukey's multiple comparison).

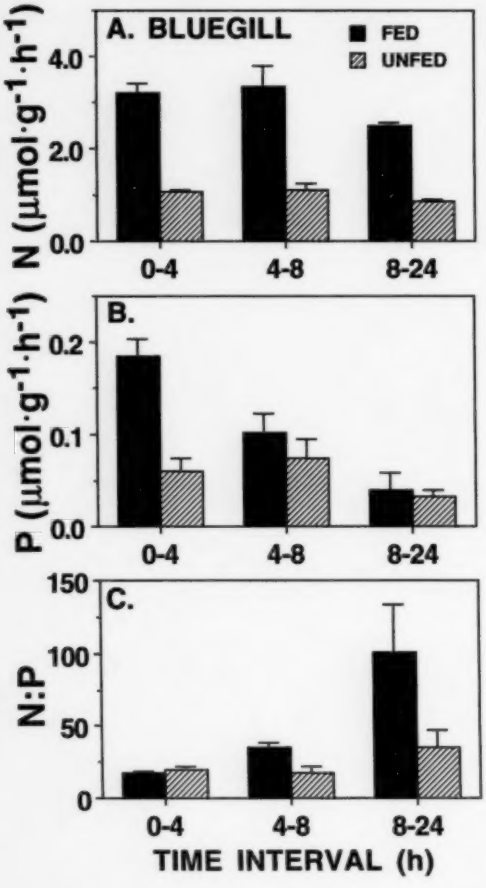
****$P \leq 0.0001$ (Tukey's multiple comparison).

time because fish excreted more N immediately after eating (0–4, 4–8 h) than at the end of the digestive process (8–24 h; Fig. 1A; time effect, $P \leq 0.01$ (Tukey $P \leq 0.01$); Table 1). Feeding and time did not interact; that is, N excretion rates remained low throughout the experiment for unfed fish but were consistently higher for fed fish (Fig. 1A; interaction effect, $P = 0.94$, Table 1).

Phosphorus excretion rates for bluegill

Mean P excretion rates ranged from 0.03 to 0.07 μmol·g^{-1}·h^{-1} for unfed bluegill (1.0–2.3 μg·g^{-1}·h^{-1}), and from 0.04 to 0.18 μmol·g^{-1}·h^{-1} for fed fish (1.2–5.7 μg·g^{-1}·h^{-1}; Fig. 1B). Overall, fed fish had higher P excretion rates than unfed fish (Fig. 1B; feeding effect, $P \leq 0.01$ (Tukey $P \leq 0.001$); Table 1). Phosphorus excretion was highest immediately after eating (0–4 h) and declined through time (Fig. 1B; time effect, $P \leq 0.01$ (Tukey $P \leq 0.001$); Table 1). The effect of time varied with feeding regime, i.e., for fed fish, P excretion rates declined markedly through 24 h, whereas for unfed fish, P excretion rates remained low throughout the experiment (Fig. 1B; interaction effect, $P \leq 0.01$ (Tukey $P \leq 0.01$); Table 1).

Fig. 1. Bluegill excretion rates of (A) ammonia-N (μmol N·g fish^{-1}·h^{-1}) and (B) soluble reactive phosphorus (μmol P·g fish^{-1}·h^{-1}), and (C) the ratio of excreted nitrogen to phosphorus (molar) through time in treatments with fish fed chironomid larvae (solid bars) and unfed fish (hatched bars). Data are given as the mean ± 1 SE; $N = 4$ or 5. The results of a two-way ANOVA testing the effects of feeding regime and time course are shown in Table 1.



N:P ratio excreted for bluegill

The mean molar ratio of excreted N to P ranged from 18:1 to 35:1 for unfed bluegill (8:1 to 16:1 by weight) and from 18:1 to 101:1 for fed fish (8:1 to 45:1 by weight; Fig. 1C). Fed fish excreted a higher N:P ratio than unfed fish (Fig. 1C; feeding effect, $P \leq 0.01$ (Tukey $P \leq 0.01$); Table 1). For fed fish, the N:P ratio increased through time (Fig. 1C; time effect, $P \leq 0.01$ (Tukey $P \leq 0.01$); Table 1), although the interaction between feeding and time was not significant (Fig. 1C; Table 1).

Nitrogen excretion rates for adult gizzard shad

Mean N excretion rates averaged 1.1 μmol·g^{-1}·h^{-1} for unfed adult gizzard shad (15.2–15.5 μg·g^{-1}·h^{-1}), and for fed fish, the N excretion rate ranged from 0.4 to 7.2 μmol·g^{-1}·h^{-1} (5.9–101.3 μg·g^{-1}·h^{-1}; Fig. 2A). Over all time periods, fish in both fed treatments excreted more N than unfed fish (Fig. 2A, All; feeding effect for all intervals, $P \leq 0.01$ (Tukey $P \leq 0.01$); Table 1). The nitrogen excretion rate of unfed fish remained constant through time (Fig. 2A, All) but for fed fish, the N excretion rate was high immediately after feeding (0–1 h) and decreased dramatically through 8 h (Fig. 2A, Early; time effect for early intervals only, $P \leq 0.01$ (Tukey $P \leq 0.0001$); Table 1). As digestion progressed (4–8, 8–24 h), N excretion rates by fish in both fed treatments were low (Fig. 2A, Early and All). The early peak and subsequent decrease in the excretion rates of fed fish were largely masked when all early intervals (0–8 h) were examined together and the change in N excretion through time was only marginally significant when the excretion of fed fish was measured only at 8 and 24 h (Fig. 2A, All; time effect for all intervals, $P = 0.06$; Table 1). However, the change in the N excretion rate of fed fish differed from the low and constant excretion rate of unfed fish (Fig. 2A, All; interaction effect for all intervals, $P = 0.02$ (Tukey $P \leq 0.05$); Table 1). Although N excretion rates varied between the two fed treatments (Fig. 2A, Early; treatment effect for early intervals only, $P \leq 0.03$ (Tukey $P \leq 0.05$); Table 1), trends through time were similar (Fig. 2A, Early; interaction effect for early intervals only, $P = 0.77$; Table 1).

Phosphorus excretion rates for adult gizzard shad

Mean P excretion rates ranged from 0.03 to 0.04 μmol·g^{-1}·h^{-1} for unfed gizzard shad (1.0–1.3 μg·g^{-1}·h^{-1}), and from 0.10 to 0.44 μmol·g^{-1}·h^{-1} for fed fish (2.0–13.7 μg·g^{-1}·h^{-1}; Fig. 2B). Overall, fed fish had higher P excretion rates than unfed fish (Fig. 2B, All; feeding effect for all intervals, $P \leq 0.01$ (Tukey $P \leq 0.0001$); Table 1). For fed fish, P excretion rates declined dramatically through time (Fig. 2B, All; time effect for all intervals, $P \leq 0.01$ (Tukey $P \leq 0.01$); Table 1). Considering only the early time intervals, across both feeding treatments, P excretion rates were highest immediately after the fish had eaten (0–1 h) and declined through time (Fig. 2B, Early; time effect for early intervals only, $P \leq 0.01$ (Tukey $P \leq 0.0001$); Table 1). Conversely, unfed gizzard shad excreted low and constant levels of P throughout the experiment (Fig. 2B, All; interaction effect for all intervals, $P = 0.03$ (Tukey $P \leq 0.001$); Table 1). Similar to the excretion rates of N, P excretion rates differed between the two fed treatments (Fig. 2B, Early; treatment effect for early intervals only, $P \leq 0.01$ (Tukey $P \leq 0.01$); Table 1), but again trends through time were similar across treatments (Fig. 2B, Early; interaction effect for early intervals only, $P = 0.26$; Table 1).

N:P excretion ratio in adult gizzard shad

The mean molar ratio of excreted N:P ranged from 33:1 to 35:1 for unfed gizzard shad (15:1 to 16:1 by weight) and from 3:1 to 35:1 for fed shad (1:1 to 16:1 by weight; Fig. 2C). Over all time periods, fed gizzard shad excreted lower N:P ratios than unfed fish (Fig. 2C, All; feeding effect for all intervals, $P \leq 0.01$ (Tukey $P \leq 0.0001$); Table 1). The N:P excretion ratio of fed fish exhibited a marginally significant decrease from 0 to 8 h (Fig. 2C,

Early; time effect for early intervals, $P \leq 0.06$; Table 1) but overall the N:P ratio increased with time, especially between 8 and 24 h (Fig. 2C, All; time effect for all intervals, $P \leq 0.01$ (Tukey $P \leq 0.001$); Table 1). Conversely, the N:P excretion ratio of unfed gizzard shad remained high and constant throughout the experiment (Fig. 2C, All; time effect for all intervals, $P \leq 0.01$ (Tukey $P \leq 0.01$); interaction effect for all intervals, $P \leq 0.01$ (Tukey $P \leq 0.01$); Table 1). The N:P excretion ratio differed between the two fed treatments (Fig. 2C, Early; treatment effect for early intervals only, $P \leq 0.01$ (Tukey $P \leq 0.001$); Table 1), but again, the trends exhibited by fish in the two fed treatments were similar through time (Fig. 2C, Early; interaction effect for early intervals only, $P = 0.47$; Table 1).

Young-of-year gizzard shad

The excretion rates of young-of-year gizzard shad showed similar trends. Young-of-year fish excreted consistently high levels of N through 8 h (Fig. 3A; time effect, $P = 0.71$; Table 1). The P excretion rates for these small fish were initially high and decreased dramatically as the experiment progressed (Fig. 3B; time effect, $P \leq 0.01$ (Tukey $P \leq 0.0001$); Table 1). As a result, young-of-year fish excreted an N:P ratio that was low immediately after feeding (0–1 h), increased by 1–2 h, and remained high through 8 h (Fig. 3C; time effect, $P = 0.03$ (Tukey $P \leq 0.05$); Table 1).

Gizzard shad size comparisons

The N excretion rates differed among size treatments ($P = 0.05$ (Tukey $P \leq 0.05$)) but not among time intervals ($P = 0.64$), although there was a suggestion of a treatment–time interaction ($P = 0.09$). In general, N excretion rates were higher for young-of-year fish, especially in the 4- to 8-h interval and especially when compared with the values for Fed-2 (Figs. 2 and 3). The P excretion rate was marginally different among treatments ($P = 0.06$) and young-of-year fish had P excretion rates intermediate to those of the adult fish in the Fed-1 and Fed-2 treatments. As in the separate analyses of time effects on young-of-year and adult gizzard shad, time since feeding had a highly significant effect on P excretion rates ($P \leq 0.01$ (Tukey $P \leq 0.001$)), although the treatment–time interaction was not significant ($P = 0.26$). Thus, in our experiments, time since feeding had a greater effect on P excretion rates than did fish size. The N:P ratio excreted was significantly affected by treatment ($P \leq 0.01$ (Tukey $P \leq 0.001$)) and time since feeding ($P = 0.01$), that is, young-of-year fish excreted at a higher N:P ratio than fish in either adult treatment in the 1- to 4- and 4- to 8-h intervals but not in the 0- to 1-h interval (interaction effect, $P = 0.02$; Figs. 2 and 3).

Electroshocking effects on excretion rates and model

Electroshocking had no statistically detectable effect on young-of-year gizzard shad excretion rates or ratio. The N excretion rate was 1.22 ± 0.23 $\mu mol \cdot g^{-1} \cdot h^{-1}$ (mean ± SE) for control fish and 1.35 ± 0.34 $\mu mol \cdot g^{-1} \cdot h^{-1}$ for electroshocked fish ($P = 0.76$), while P excretion rate was 0.078 ± 0.014 $\mu mol \cdot g^{-1} \cdot h^{-1}$ for control fish and 0.063 ± 0.012 $\mu mol \cdot g^{-1} \cdot h^{-1}$ for electroshocked fish ($P = 0.44$). The molar N:P ratio excreted for control fish was 23.17 ± 5.03 and 18.45 ± 4.65 for electroshocked fish ($P = 0.51$). Thus, it appears that this method of capture did not bias our results for field-collected gizzard shad. For P, predictions from the model agreed well with that from experiments.

Fig. 2. Adult gizzard shad excretion rates of (A) ammonia-N ($\mu mol\ N \cdot g\ fish^{-1} \cdot h^{-1}$) and (B) soluble reactive phosphorus ($\mu mol\ P \cdot g\ fish^{-1} \cdot h^{-1}$), and (C) the ratio of excreted nitrogen to phosphorus (molar) through 24 h in treatments with fish fed Acton Lake sediments (black and gray solid bars) and unfed fish (hatched bars). For gizzard shad, Fed-1 and Fed-2 represent two separate feeding experiments conducted in May and June, 1992. On the left side of each panel, all early time intervals are shown for the fed fish (0–1, 1–4, 4–8 h) to depict the shape of the initial response. On the right side of each panel, fed and unfed fish are compared over the entire experiment (0–8, 8–24 h). Data are given as the mean ± 1 SE; $N = 3$–6. The results of a two-way ANOVA testing the effects of feeding regime and time course for all intervals and for fed fish during the early intervals only are shown in Table 1.

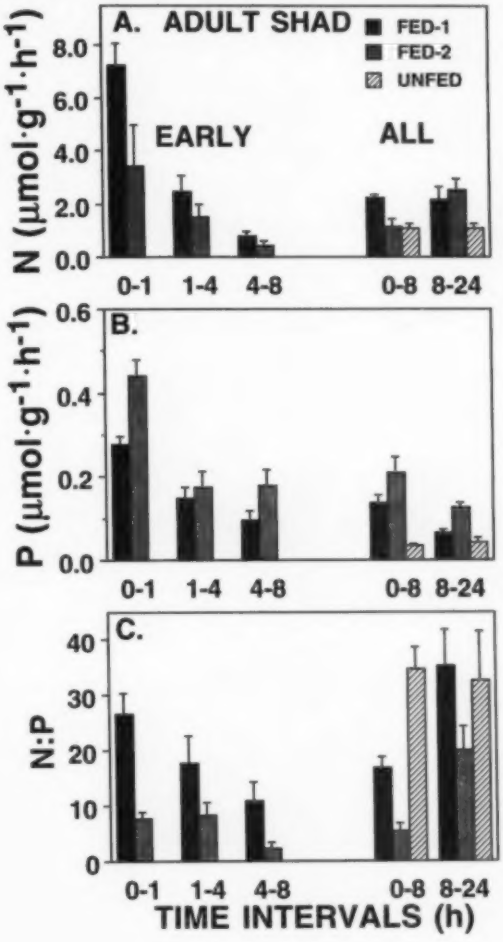
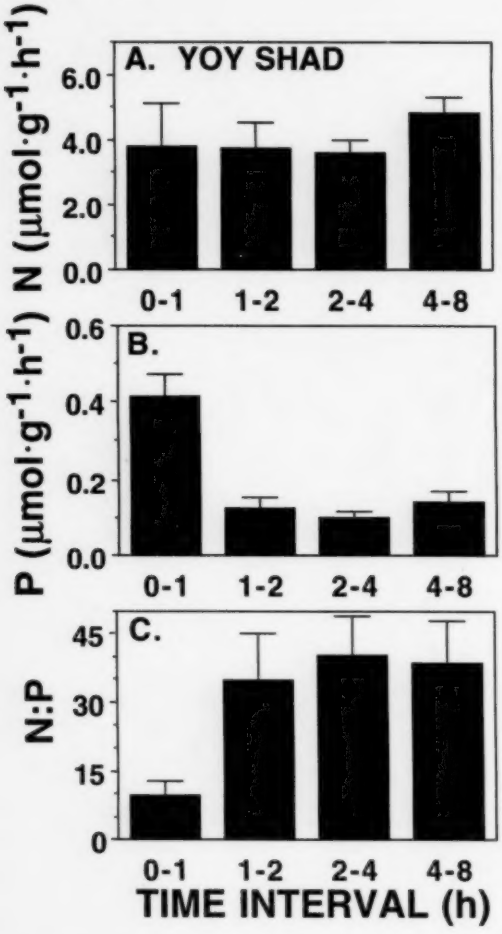


Fig. 3. Young-of-year gizzard shad excretion rates of (A) ammonia-N (μmol N·g fish^{-1}·h^{-1}) and (B) soluble reactive phosphorus (μmol P·g fish^{-1}·h^{-1}), and (C) ratio of excreted nitrogen to phosphorus (molar) for fish allowed to feed on Acton Lake sediments through 8 h in July 1993. Data are given as the mean ± 1 SE; $N = 4$. The results of an ANOVA testing the effects of time are shown in Table 1.



Measured P excretion rates were 18 and 2.5% lower than predicted by the model in the Fed-1 and Fed-2 experiments, respectively, and the mean of the modelled P excretion rate was within 10% of the mean of the experimentally derived rates (Table 2). For N, agreement was not as strong, yet still reasonable. Measured N excretion rates were 39% higher (Fed-1) and 36% lower (Fed-2) than modelled rates, although the mean of N for the model was within 2% of that predicted (Table 2).

Discussion

Importance of nutrient excretion by fish

In lakes, the relative contribution of nutrients excreted by fish, zooplankton, and external loading has been debated. In Lake Wingra, Wisconsin, fish regenerate an insignificant amount of P compared with zooplankton (Kitchell et al. 1975). Similarly, in Lake Memphremagog, located in Quebec and Vermont, the amount of P recycled by adult yellow perch (*Perca flavescens*), 0.33% of the seston P demand, is insignificant relative to the 12% regenerated by zooplankton (Nakashima and Leggett 1980). However, in other studies, P recycling by fish equalled or exceeded that regenerated by either zooplankton (Vanni and Findlay 1990; Carpenter et al. 1992*a*, 1992*b*; Schindler et al. 1993) or external loading (Shapiro et al. 1983; Brabrand et al. 1990). Moreover, in other systems, nutrient excretion by fish is an important source of P (Lamarra 1975; Mazumber et al. 1988; Brabrand et al. 1990; Kraft 1992; Schindler et al. 1993), increases primary productivity (Lamarra 1975; Brabrand et al. 1990; Schindler et al. 1993), and alters phytoplankton species composition (Brabrand et al. 1990; Vanni and Findlay 1990).

Why do these studies differ in their conclusions about the importance of nutrient excretion by fish? Ecological and behavioral considerations such as feeding habits, fish size, diel movements, system size, and fish community structure can introduce variations in nutrient regeneration rates. For example, sediment-feeding roach (*Rutilus rutilus*) excrete more P than planktivorous roach (Brabrand et al. 1990). In addition, small fish may recycle more P than large fish on a mass-specific basis (Lamarra 1975; Brabrand et al. 1990; Kraft 1992; Carpenter et al. 1992*a*; Schindler et al. 1993) and young-of-year yellow perch contribute more to limnetic P cycling than older fish (Kraft 1992), probably because of the inverse relationship between size and specific metabolic rate. Fish movements also affect the relative importance of nutrient excretion by fish (Carpenter et al. 1992*a*). For example, roach that migrate daily between limnetic and littoral zones may maximize the contribution of nutrient excretion by fish by consuming littoral zone sediments high in P and, through excretion, transporting these nutrients to the limnetic zone where they were previously unavailable to phytoplankton (Brabrand et al. 1990).

Lake size, food web structure, and fish population density cause further across-system variation. Fish nutrient regeneration may be more important in small lakes (Nakashima and Leggett 1980) and in lakes dominated by planktivores (Carpenter et al. 1992*a*, 1992*b*; Schindler et al. 1995). For example, in Peter Lake, Michigan, a planktivore-dominated system where the majority of the standing crop is fish, 85% of the P recycled was derived from nutrient excretion by fish, three times more P was incorporated into primary production, and algal biomass was 30% higher than in West Long Lake, Michigan, a piscivore-dominated system (Schindler et al. 1993). In addition, as fish density increases, the importance of nutrient excretion by fish increases, e.g., roach biomass in the lake studied by Brabrand et al. (1990) was 20 times higher than yellow perch density in Lake Memphremagog, the system for which Nakashima and Leggett (1980) concluded that nutrient regeneration by fish was unimportant. Thus, fish ecology, behavior, and community structure explain some of the ambiguity surrounding the importance of nutrient recycling by fish.

Table 2. Comparison of modelled and observed N and P excretion rates and percentage by which observed values exceeded those predicted by our model (O/M) for adult gizzard shad.

	N excretion rate			P excretion rate		
Experiment	Model	Observed	O/M (%)	Model	Observed	O/M (%)
Fed-1						
$\mu mol \cdot g^{-1} \cdot d^{-1}$	87.8	121.9	>39	6.0	4.9	<18
$\mu g \cdot g^{-1} \cdot d^{-1}$	1229	1701		187	151	
Fed-2						
$\mu mol \cdot g^{-1} \cdot d^{-1}$	108.1	69.4	<36	7.9	7.7	<2.5
$\mu g \cdot g^{-1} \cdot d^{-1}$	1514	972		244	238	
Mean						
$\mu mol \cdot g^{-1} \cdot d^{-1}$	97.9	95.7	<2	7.0	6.3	<10
$\mu g \cdot g^{-1} \cdot d^{-1}$	1371	1340		216	195	

Note: Predictions were made using the ecological parameters associated with both the Fed-1 and Fed-2 experiments. Details of the model are described in the text.

Comparison of excretion rates with other studies

In this study, P excretion rates of unfed fish, 0.03–0.07 $\mu mol \cdot g\ fish^{-1} \cdot h^{-1}$ (1.0–2.3 $\mu g \cdot g\ fish^{-1} \cdot h^{-1}$), and fed fish, 0.04–0.44 $\mu mol \cdot g\ fish^{-1} \cdot h^{-1}$ (1.2–13.7 $\mu g \cdot g\ fish^{-1} \cdot h^{-1}$), were within the range reported in other studies, 0.03–0.15 $\mu mol \cdot g\ fish^{-1} \cdot h^{-1}$ (0.8–4.7 $\mu g \cdot g\ fish^{-1} \cdot h^{-1}$; Lamarra 1975), 0.03–0.10 $\mu mol \cdot g\ fish^{-1} \cdot h^{-1}$ (0.78–3.16 μg; Nakashima and Leggett 1980), and 0.06–0.25 $\mu mol \cdot g\ fish^{-1} \cdot h^{-1}$ (2.0–7.6 $\mu g \cdot g\ fish^{-1} \cdot h^{-1}$; Brabrand et al. 1990). Nitrogen excretion has been measured less often by others than P excretion, at least in fish feeding on natural prey. Few studies have measured the N:P ratio at which fish excrete and none examined the effect of both diet and time since feeding. Brabrand et al. (1990) found the N:P excretion ratio of roach to average 27:1 molar (12:1 by weight) and Kraft (1992), using a bioenergetics – mass balance model, found that the N:P excretion ratio of yellow perch could vary from 29:1 to 104:1 molar (13:1 to 47:1 by weight). The N:P excretion ratios of fish in our study were 3:1 to 101:1 molar (1:2 to 45:1 by weight) for fed fish and 18:1 to 35:1 (8:1 to 16:1 by weight) for unfed fish. Thus, bluegill in our study generally excreted at N:P ratios within the range reported by others; however, gizzard shad that had fed recently (within the last 8 h) sometimes excreted at considerably lower N:P ratios.

Effect of feeding, time, species, and size

In our experiments, feeding significantly increased the amount of N and P excreted by both bluegill and adult gizzard shad, although the effect differed between species. Fed bluegill excreted 3.0 times more N and 2.0 times more P than unfed fish, resulting in a higher N:P ratio for fed bluegill. Fed adult gizzard shad excreted 2.3 times more N but 4.8 times more P than unfed shad; consequently, feeding reduced the N:P ratio excreted by gizzard shad to less than half. Thus, for both species, the N:P ratios differed with feeding, but feeding increased the N:P excretion ratio for one species and decreased it for the other species.

In our experiments, the pattern of excretion also changed with time. N and P excretion rates for all species and sizes were highest early in the digestive process. The P excretion rate decreased more rapidly through time than the N excretion rate, resulting in an excreted N:P ratio that increased with time since feeding. Our experiments were not an explicit test of species-specific differences because fish biomass, density, and diet differed in the two sets of experiments. However, the effect of feeding regime on excretion rates and N:P excretion ratio produced three similar between-species trends. First, for both bluegill and adult gizzard shad, excretion rates of N and P by unfed fish were low, constant, and numerically similar between species. Second, feeding increased the excretion rates of N and P for both species. Third, for both species when fed, the N:P excretion ratio increased with the passage of time since feeding. Both adult and young-of-year gizzard shad showed similar patterns of excretion through time; however, young-of-year fish excreted higher levels of N through 8 h than adults, resulting in a higher N:P excretion ratio for these small fish. Thus, the age structure of a fish population and the production of strong year-classes, as well as feeding, time since feeding, and species, may be important in nutrient recycling.

Comparison of model output versus measured results

For P, predictions of the model agreed quite well with the results of the experiments. For N, agreement was not as strong, yet still reasonable. Interestingly, the mean of the modelled N excretion rates agreed quite well with the mean of the experimentally derived rates. N excretion rates probably depend more on metabolic parameters (e.g., protein catabolism) than do P excretion rates; hence, a simple mass balance approach such as this may be more valid for P than N.

Within-study variations

In our experiments, several problems existed. First, unfed fish were allowed to recover from the dipnet handling

whereas fed fish were not. Although we did not quantify the effects of handling stress on excretion, we did find that collection by electrofishing had no effect. In hatchery cutthroat trout, *Salmo clarkii*, the levels of plasma cortisol, a physiological stress indicator, change less in response to a 30-s dipnet handling than to electroshocking (Mesa and Schreck 1989). Because handling has less of an effect on cortisol levels than electroshocking and electroshocking does not affect nutrient excretion, lack of acclimation to handling in the fed fish treatment should not have affected excretion in our experiments. In addition, predicted excretion rates from our model agreed with the measured rates for fed gizzard shad, and feeding affected bluegill and gizzard shad similarly. Thus, methodological differences between the experiments on fed and unfed gizzard shad likely did not affect our results.

Second, the absolute concentration of nutrients excreted by adult gizzard shad in the two fed treatments differed. Natural variability in opportunistically feeding, field-collected fish such as gizzard shad may be inescapable. In our study, differences in fish sizes, temperature, ration, and food composition existed. In the Fed-1 treatment, we used smaller fish that excreted more N, less P, and a higher N:P ratio than the fish in the Fed-2 treatment. Even though the absolute levels of excretion differed between the Fed-1 and Fed-2 fish, these two treatments changed similarly through time and mirrored trends through time found for excretion by fed young-of-year gizzard shad and fed bluegill.

Third, before our experiments began we replaced half of the water in each test tank with deionized water. Diffusion in the form of NH_3 is responsible for most ammonia efflux and a correlation exists between ammonia excretion and the internal–external ammonia gradient (Avella and Bornancin 1989). Although adding deionized water to our fish tanks may have reduced the amount of ammonia and P in the ambient water, the amount of ammonia in lake water is naturally low, and thus decreasing the number of ammonia ions in the experimental tank via the addition of deionized water is unlikely to significantly affect either the N or P diffusion gradient. Replacing existing water with deionized water may also affect the Na^+–NH_4^+ pump through which sodium is taken into the cell in exchange for ammonia release (Payan 1978; Avella and Bornancin 1989). However, the addition of deionized water, if anything, would reduce the release of ammonia via this mechanism and thus underestimate the effect of feeding. Thus, the trend we observed likely is related to feeding regime and not to an osmotic challenge associated with methodology.

Implications for phytoplankton communities and nutrient fluxes

Excretion of nutrients by fish can increase algal biomass (Lamarra 1975; Vanni and Findlay 1990; Brabrand et al. 1990; Schindler et al. 1993) and may alter the species composition of algae by altering competitive balance between species. Specifically, algal species favored by high nutrient supply rates, such as many greens and blue-greens, may be favored when the nutrient excretion rates of fish are high (Andersson et al. 1978; Reinertsen et al. 1986; Vanni and Findlay 1990).

Nutrient excretion by fish also may have important effects on phytoplankton communities and water quality because the supplied N:P ratio can be a critical determinant of phytoplankton community structure (Smith 1983; Elser et al. 1988; Sterner 1990; Helal and Culver 1991). Nitrogen-fixing blue-green algae often dominate lakes when N:P ratios are low (Tilman et al. 1982; Smith 1983). Smith (1983) found that blue-greens are relatively rare when the N:P ratio is >64:1 molar (29:1 by mass) and can dominate algal biomass when nutrient levels are below this ratio. In our experiments, the N:P excretion ratios ranged from 4:1 to 101:1 molar (2:1 to 45:1 by mass). Because these N:P excretion ratios bracket the Redfield ratio as well as the 64:1 molar (29:1 by mass) ratio deemed critical by Smith (1983), nutrient excretion by fish could either favor or inhibit blue-green algae. If the ratios of nutrients excreted by fish differ from the nutrient content of the prey, as they do for zooplankton (Elser et al. 1988; Sterner 1990; Sterner et al. 1992), another layer of complexity will be added to the study of nutrient budgets.

Fish feeding behavior and movement patterns can affect the transport of nutrients between littoral–benthic and pelagic habitats (Lamarra 1975; Schindler et al. 1995; Vanni 1995), and fish feeding on benthic prey but excreting nutrients into open waters may represent a substantial vector for nutrient transport into epilimnetic waters. Because it is often difficult to balance nutrient budgets in lakes (e.g., Caraco et al. 1988, 1992), transport of nutrients by fish should be considered in efforts to construct such budgets. The timing of nutrient excretion by fish may also be important, and may be affected by feeding behavior and movement. Temporal variation in nutrient supply rate may affect phytoplankton community structure by favoring species adapted to sequester nutrient pulses and by facilitating coexistence (Sommer 1989). Because time since feeding influences the rates and ratios of nutrients excreted, and fish often show diel feeding patterns and movement, they may excrete nutrients at rates that vary substantially over a 24-h period. Such pulsed nutrient excretion may affect phytoplankton communities (Reinertsen et al. 1986). In summary, food habits, fish size, diel feeding patterns, habitat use, food web configuration, and between-habitat movements may affect patterns of nutrient regeneration and availability and thereby contribute significantly to variability in nutrient budgets, phytoplankton community structure, and the outcome of biomanipulation experiments.

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Physiological responses and depression of humoral components of the immune system in gilthead sea bream (*Sparus aurata*) following daily acute stress

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Abstract: Changes in the immune system indicators serum hemagglutinating activity, complement levels, antibody titer, and lymphocyte number and in the plasma levels of cortisol and glucose in the gilthead sea bream (*Sparus aurata*) indicated that stress was induced when fish were chased daily for 8 min with a hand-held net for 16 days. Plasma levels of cortisol and glucose were elevated after 1 day and cortisol remained above prestress levels throughout the experiment. The immune system was altered as indicated by lymphocytopenia and decreases in hemolytic activity, agglutination capacity, and antibody titer. As these results show, for the first time, that the complement system is depressed after stress, a routine complement analysis is suggested as an additional technique for assessing the health status of fish.

Résumé : La réponse de stress a été mésurée sur la dorade (*Sparus aurata*) après 16 jours de poursuite régulière (8 min/jour). On a analysé le cortisol et la glucose plasmatiques et des réponses immunologiques, comme l'activité hémagglutinante du sérum, l'action hémolysante du complément, la titration des anticorps et le nombre de lymphocytes circulants. Les résultats montrent une augmentation du cortisol et de la glucose après le premier jour, laquelle continue pour le cortisol jusqu'à la fin de l'expériment, où on trouve une stabilisation. Toutes les réponses immunologiques sont affectées. On trouve lymphocytopenie et une baisse de l'activité hémolitique, de la capacité d'agglutination et de la titration des anticorps. Puisque ces résultats indiquent l'inhibition du système du complément par le stress, on suggère l'addition des analyses du complément à l'ensemble des autres indicateurs sanitaires des poissons.

Introduction

Fish farms, hatcheries, and other aquaculture facilities may often suffer epizootic episodes leading to severe diseases and losses because of stress (Flos et al. 1990). Stressful conditions may lead to a decline in the effectiveness of the immune system, components of which become depressed (Ellsaesser and Clem 1986; Maule et al. 1989). Thus, fish under such conditions are more susceptible to pathogens (Maule et al. 1989; Pickering and Pottinger 1989). Although the precise mechanism(s) of the effect of stress on the immune system is (are) still under study, changes observed to date have often been associated with increases in circulating corticosteroid levels, which affect immune function in both mammals and fish (Wechsler et al. 1986; Maule et al. 1987; Bateman et al. 1989).

Immunological parameters used to assess the health status of fish have mainly been based on variations in leucocrit, differential white cell count, antibody titration, phagocytic activity of macrophages, and the oxidative activity of neutrophils. Depression of the antibody response (Anderson 1990), reduction of splenic antibody-secreting cells (Mazur and Iwama 1993), and lymphocytopenia (Pickering 1984) have been described in fish experiencing stress. Decreases in the phagocytic index and respiratory burst activity of phagocytes have also been described as indicators of immunosuppression (Stave and Roberson 1985; Angelidis et al. 1987). Such responses are important tools for monitoring the immunocompetence of fish. Teleost fish possess a complement system similar to that of mammals, in addition to the antibody and phagocytic response (Sakai 1992; Lambris et al. 1994). In the few studies carried out to date, fish complement, particularly the alternative complement pathway (ACP), has been shown to be present at higher levels than in mammals (Sunyer and Tort 1995). At present, no data are available concerning the effects of stress on the complement system in fish.

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Fish sera normally have hemagglutinating activity, which is usually related to the presence of lectins or hemagglutinins. These molecules have been reported to play a role as a nonspecific defense mechanism (Ingram 1980). The capacity of hemagglutinins to agglutinate different pathogens and their ability to act as opsonins have been reported elsewhere (Alexander and Ingram 1992). However, no study has reported variations in hemagglutinating activity under conditions of stress in fish.

Responses to acute stress have been well characterized in both mammals (Natelson et al. 1981) and fish (Barton and Iwama 1991). Hormonal responses in fish include an initial sharp and dramatic increase in plasma levels of cortisol and catecholamines (within 5–10 min) and recovery within a few hours (Pickering et al. 1987). Chronic or continuous disturbance, which is common in aquaculture operations, results in the induction of a wide range of responses and systems, eventually leading to adaptation. Repeated disturbances are often less well defined even in mammals and include a wide variety of modalities depending on the factors involved, e.g., periodicity, regularity, and type and intensity of stressor (Martì et al. 1993). The term chronic stress has been used in mammals to refer to various treatments that differ in either the duration or total length of exposure (see Baum et al. (1993) for a discussion in mammals). In fish, chronic stress has often been associated with crowding and confinement, which involve the continuous presence of a stressor. Our study focused on physiological responses caused by an intermittent stressor, which consisted of the same disturbance applied with regular periodicity, with the same intensity and duration. These types of stressors are common in aquaculture operations, as procedures such as cleaning, feeding, or grading are often regular and periodic. However, little work has been done on the responses of fish to this type of stressor. One previous study used the term daily acute stress (Barton et al. 1987), and we use the same expression here.

In this study we describe the changes in complement and hemagglutinating activity of serum in gilthead sea bream (*Sparus aurata*) during exposure to intermittent handling. In addition, antibody titers and the number of circulating lymphocytes were measured. Plasma levels of glucose and cortisol were measured to monitor the metabolic and endocrine responses to handling and to verify that the fish had been stressed.

Materials and methods

Fish

Gilthead sea bream, obtained from the AQUADELT S.A. fish farm in the Ebre delta (eastern Spain), were acclimated for 1 month in 400-L Fiberglas laboratory tanks before the beginning of experiment. During that time, fish were maintained in a closed seawater circulation system equipped with physical and biological filters. Aeration and ammonia and nitrite levels were monitored continually. Water temperature was kept at 12°C, salinity at 3.8%, and photoperiod constant at 12 h light : 12 h dark. Fish were fed a ration of 1% body weight daily with extruded pellets (Gallina Blanca – Purina, Aquaculture Division). Fish weight at the beginning of experiment was 220 ± 20 g (mean ± SE).

Experimental conditions and sampling procedure

After acclimation, 30 fish were chased with a hand-held net in the tank for 8 min every day for the 16 consecutive days of the experiment. Fish were sampled at 1, 3, 7, and 16 days. The samples were obtained before the chasing period to avoid effects of the immediate stress reaction. Twenty-seven undisturbed fish of the same size held in tanks with the same water conditions, and in the same aquarium room, served as controls. Sampling consisted of rapidly capturing the fish and extracting 0.8 mL of blood from the caudal vessels with a syringe. Sampled fish were not reused. Each sampling day, control fish were processed first, and stressed fish were sampled later to avoid possible effects of an acute stress response caused by sampling. After each sampling, the water level was adjusted to maintain a constant density of fish. A portion of blood was placed in cold heparinized tubes for cell counting and plasma analysis. The remaining blood was allowed to clot at 4°C for 2 h. The serum was then separated by 10-min centrifugation and several aliquots were stored at −80°C to retain complement activity.

Buffers and rabbit blood preparation

The following buffers were used: (*i*) GVB (isotonic veronal-buffered saline), pH 7.3, containing 0.1% gelatin, and EDTA-GVB, which was the same buffer but containing 20 mM EDTA. To measure the alternative complement pathway (ACP), serum samples were diluted in GVB (pH 7.3) containing 10 mM Mg^{2+} and 10 mM EGTA (Mg-EGTA-GVB). The classical complement pathway (CCP) and spontaneous hemolytic activity (SH) were determined by diluting samples in GVB containing 10 mM Mg^{2+} and 2 mM Ca^{2+} (Mg-Ca-GVB). Rabbit red blood cells (RaRBC) were used for the immune tests and prepared as follows. Blood was withdrawn from the ear artery of a rabbit and placed into heparinized tubes. Red blood cells were washed four times in GVB and resuspended to a concentration of 2.5×10^8 cells/mL with the appropriate buffer. Unsensitized RaRBC were used for the ACP and SH titration. For the CCP titration, RaRBC were sensitized with a 1:100 dilution of heat-deactivated fish antiserum raised against RaRBC. This dilution was previously found to be the highest one at which the maximal rate of lysis of hemolysin-bound RaRBC by sea bream complement could be achieved (Sunyer and Tort 1994).

Hemolytic assays

Three hemolytic activities were measured: ACP, CCP, and SH. Twenty-five microlitres of RaRBC suspension (unsensitized for the ACP and SH activities and sensitized RaRBC for CCP activity) was added to 100 μL of serially diluted sea bream serum in the appropriate buffer. The tubes were incubated at 20–22°C for 100 min with regular shaking. The reaction was stopped by adding 1 mL of cold EDTA-GVB. The tubes were then centrifuged and the extent of hemolysis was estimated by measuring the optical density of the supernatant at 414 nm (OD_{414}). Total or 100% hemolysis is given by the optical reading of the supernatant from 25 μL of the used RaRBC suspension added to 1100 μL of distilled water. The reciprocals of the serum dilution causing 50% lysis of RaRBC were designated as ACH_{50},

SH_{50}, and CH_{50} titers for the ACP, SH, and CCP activities, respectively. Results are presented as ACH_{50}, CH_{50}, and SH_{50} units per millilitre. The value of Y (percentage of RaRBC lysed at each dilution) was defined as $Y = (Abs_{414}A - (Abs_{414}B + Abs_{414}C)) / (Abs_{414}D - Abs_{414}B)$, where A is the supernatant of the test serum dilution, B is the supernatant of the buffer containing RBC without the serum, C is the serum dilution without the RBC added, and D is 100% lysis. Y values were transformed according to the Van Krogh equation. Thus, the values $Y/(1 - Y)$ and the reciprocal of serum dilutions were plotted on log–log paper and the values of ACH_{50}, SH_{50}, and CH_{50} ($Y = 0.5$ or $Y/(1 - Y) = 1$) were determined from the graph. Serum dilutions resulting in >90 or <15% were excluded from the calculation.

Procurement of anti-C3 antibody

The anti-sea bream antibody was obtained as described by Sunyer and Tort (1995). Because C3b binds covalently to zymosan particles, highly specific antibodies can be obtained by injecting zymosan–C3b particles into a rabbit. Accordingly, sea bream serum was incubated with prewashed zymosan at 5 mg/mL at room temperature for 100 min. Zymosan was pelleted and extensively washed with 0.5 M NaCl in the presence of 1% SDS. Zymosan–C3b particles were emulsified in 2 mL of complete Freund's adjuvant. Two millilitres of the emulsified solution was injected subcutaneously and intramuscularly into a rabbit. A week later, the injection was repeated in incomplete Freund's adjuvant. Ten days later, blood was collected from the rabbit. The specificity of the antiserum was measured by immunoelectrophoresis and crossed immunoelectrophoresis, and by its capacity to inhibit the hemolytic activity of sea bream serum (Sunyer and Tort 1995).

Determination of C3 levels

Levels of C3 were measured by single radial immunodiffusion (SRID). Briefly, 1% agarose and 0.25% NaN_3 in phosphate-buffered saline (PBS) containing 10 mM EDTA (pH 7.4) was heated to 55°C and anti-sea bream C3 (final concentration 5% (v/v)) was added with stirring. Two millilitres of the mixture was added for each glass plate (7.5 × 2.5 cm). Three millimetre diameter holes were punched into agarose with 2.5-cm spaces between the holes. Six microlitres of sea bream serum was dispensed into each well. Plates were incubated for 18 h at room temperature. The ring diameters (D^2) were measured to the nearest 0.1 mm.

Hemagglutination assay

Hemagglutination assays were performed in micro V-plates. Serial dilutions of each serum sample were made in 25 µL of EDTA-GVB. Twenty-five microlitres of a suspension of washed RaRBC containing 2% cells in the same buffer was added to each well. The microplates were allowed to stand for 1 h at room temperature and macroscopic agglutination was recorded. Results are given as $\log_2$ values of the final serum dilution giving agglutination.

Measurement of total antibody titer by enzyme-linked immunosorbent assay

Plates of 96 wells were coated with 100 µL PBS with Na_2CO_3–glutaraldehyde buffer (coating buffer) per well for 2 h at 37°C in a humid chamber. The composition of the PBS was 8.5 g NaCl/L with 2 mM $Na_2PO_4{\cdot}2H_2O$ and 3 mM Na_2HPO_4. The plates were washed in the same PBS containing 0.05% (v/v) Tween 20. One hundred microlitres of each sea bream serum sample was serially diluted in a Na_2CO_3 buffer and incubated for 2 h at 37°C. Plates were washed three times in the same buffer. Wells were saturated with 200 µL of PBS containing 0.1% (v/v) Tween 20 and 0.5% (v/v) bovine serum albumin (BSA), for 1 h at room temperature. After washing, 100 µL per well of 1:500 monoclonal antibody against sea bream immunoglobulin (Navarro et al. 1993) in PBS containing 0.1% (v/v) Tween 20 and 0.2% (v/v) BSA was incubated overnight at 4°C. Then, wells were washed and incubated with 100 µL of 1:2000 alkaline goat anti-mouse antibody conjugated with alkaline phosphatase for 2 h at 37°C. Wells were washed in PBS containing 0.1% (v/v) Tween 20 and incubated with 100 µL of 0.75 mg 1,4-nitrophenol phosphate/mL in substrate buffer. The reaction was stopped by adding 50 µL of a 2.5 M H_2SO_4 solution per well. Plates were measured by an enzyme-linked immunosorbent assay (ELISA) read at 492 nm.

Erythrocyte and lymphocyte count

Erythrocytes were counted in a cell counter (Laboratory Instruments) after blood dilution. Lymphocyte numbers were determined under the microscope in a Neubauer hemocytometer after diluting the blood 1:20 with Türck liquid (methylene blue in glacial acetic acid and deionized water). Because of the morphologic features of these cells, lymphocytes were distinguishable and they were counted as reported elsewhere (Houston 1990).

Glucose and cortisol determination

After a portion of blood was removed for hematological analysis, the remaining blood was centrifuged and the plasma analyzed for glucose and cortisol. Glucose was measured using a colorimetric test (Boehringer Mannheim) based on the glucose oxidase reaction. Plasma cortisol was determined by radioimmunoassay using the trypsin–antitrypsin method (Saltarelli and Millgrom 1979; Armario and Castellanos 1984). This method has been validated in our laboratory for sea bream and other fish species. Corticosteroid-binding globulin was hydrolyzed using trypsin (Boehringer Mannheim). Samples were analyzed in duplicate. [1,2,6,7-^{3}H]Cortisol (Amersham International) and antiserum raised in donkeys against serum albumin conjugated cortisol-3-(*O*-carboxymethyl)oxine–bovine (Bio-Clin) were used. Cross reaction was 100% with cortisol and 0.36% with 17 α-hydroxyprogesterone. Good parallelism was found between different dilutions of the samples and the standard curve. Sensitivity was 12.5 pg/tube and recovery ranged from 94 to 105%. The intraassay coefficient of variation was 4.3%. All samples were processed at the same time to eliminate interassay variations.

Statistics

For all parameters, homogeneity of variances (Cochran and Bartlett–Box tests) was checked. A one-way analysis of variance (ANOVA) was applied to determine significant differences between controls at the different sampling times. Where there were no differences, the control groups were

Table 1. Hemolytic activities of the alternative complement pathway (ACH_{50}), classical complement pathway (CH_{50}), spontaneous hemolytic activity (SH_{50}), and C3 levels (D^2), lymphocyte numbers, and agglutination and antibody titers.

	ACH_{50} (units/mL)	SH_{50} (units/mL)	CH_{50} (units/mL)	D^2 (mm)	Lymphocytes (no./μL, $\times 10^6$)	Agglutination titer	Total antibody titer
Control	177±23	277±38	408±95	145±18	32±9.2	13.2±1.7	1430±26
1 day	173±17	298±24	470±118	150±21	24±8.0	14.2±0.4	1608±265
3 days	172±13	258±36	340±73	139±17	22±3.0*	11.3±1.5*	1441±54
7 days	199±21	293±43	446±74	162±17	27±10.1	10.7±2.4*	1080±304
16 days	124±34*	193±35*	234±73*	125±17*	22±10.3*	10.5±1.3*	827±241*

Note: Values are given as the mean ± SE. Duplicates were made for each serum titration.
*Significantly different from the control value ($p < 0.05$).

pooled and used as control values. ANOVA was also used to assess the presence of significant variation factors, followed by Duncan's multiple-range test to determine the specific differences between groups. When nonhomogeneous variances were encountered (agglutination and plasma cortisol), nonparametric Mann–Whitney and Kruskal–Wallis tests were used. In all cases, $p < 0.05$ was the accepted significance level. Regression and correlation tests were also used.

Results

The three hemolytic activities followed a similar variation pattern (Table 1). Values for controls did not vary significantly among the 4 days of sampling. Thus, control values were pooled and were 177 ± 23 ACH_{50} units/mL, 277 ± 38 SH_{50} units/mL, and 408 ± 95 CH_{50} units/mL for the ACP, SH, and CCP activities, respectively. Values for the 1st, 3rd, and 7th extraction days were not statistically different from those of controls. All three hemolytic activities were reduced to a very significant extent after 16 days of stress (to 124, 193, and 234 units/mL, respectively).

The changes in C3 levels in serum were assessed by comparing the immunodiffusion precipitin reactions from controls with those of stressed fish. The D^2 values for controls were not significantly different for the 4 extraction days. The D^2 values in nonstressed fish were 145 ± 18 mm. The D^2 values followed a variation pattern similar to that of the hemolytic activities. The first three stressed groups did not show statistically significant changes whereas the fourth group showed a significant reduction to 125 ± 17 mm (Table 1).

The hemagglutination titers for controls did not significantly differ within the four samples and thus were pooled to create a single control value (13.2 ± 1.7). The hemagglutination titers decreased significantly after 3 days and remained depressed thereafter (Table 1). As with the other parameters, no significant differences in the antibody titer were found among controls for the 4 extraction days. Thus, values from all controls were pooled, resulting in a final titer of 1430 ± 26. On the 1st, 3rd, and 7th extraction days, values were not statistically different from those of controls, but on day 16, antibody titers dropped significantly to 827 ± 241 (Table 1).

No correlation was evident between ACH_{50} and CH_{50} control values. Nevertheless, a significant correlation was found between these two titers for the stressed fish ($r^2 = 0.71$, $p < 0.001$). Correlation was found between ACH_{50} and SH_{50} titers in both control ($r^2 = 0.72$, $p < 0.001$) and stressed fishes ($r^2 = 0.84$, $p < 0.001$). A correlation was also found between control SH_{50} and CH_{50} titers ($r^2 = 0.667$, $p < 0.001$) and between the SH_{50} and CH_{50} values of stressed animals ($r^2 = 0.74$, $p < 0.001$). In a comparison between C3 levels and the hemolytic activities, statistical significance was found between D^2 values and ACH_{50} in stressed fish ($r^2 = 0.678$, $p < 0.001$) but not in controls. Moreover, significant correlations were found between D^2 and SH_{50} for control ($r^2 = 0.666$, $p < 0.001$) and stressed fish ($r^2 = 0.658$, $p < 0.001$) and also between D^2 and CH_{50} for control ($r^2 = 0.70$, $p < 0.001$) and stressed fish ($r^2 = 0.70$, $p < 0.001$).

Lymphocyte numbers decreased significantly after 3 days of daily stress (Table 1). No differences from control values were observed at day 7 but a further significant decrease was found at day 16 (Table 1).

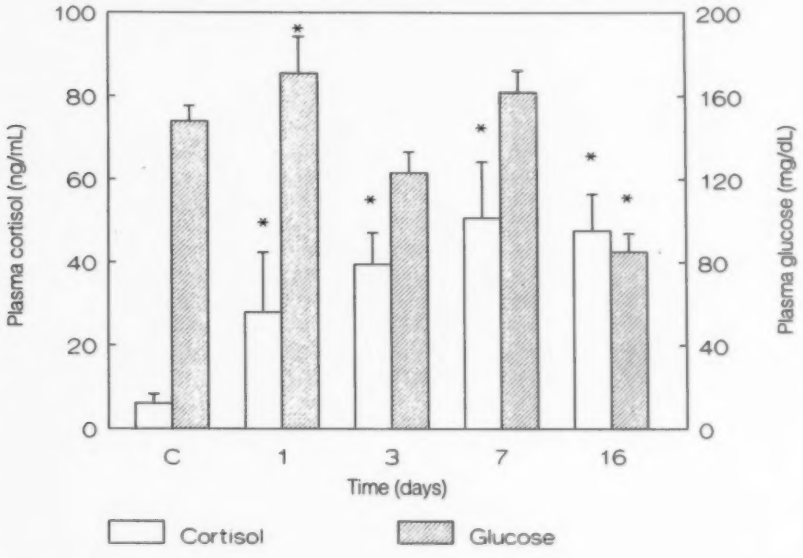
As no significant differences in plasma cortisol were found among control groups, the values were pooled to serve as the control group. Plasma cortisol values were significantly higher in all stressed groups, with levels 4 to 8 times higher than for controls (Fig. 1). A significant transient increase in plasma glucose was recorded after 1 day and no changes were detected thereafter, except for the 16-day stressed group (Fig. 1).

Discussion

Complement plays a very important role in host defense against infectious agents (bacteria, protozoans, viruses), and in the inflammatory response (Lambris 1989). Despite this, complement has not been used as a parameter to assess stress-induced immunosuppression in fish. Nevertheless, it has been reported that when coho salmon (*Oncorhynchus kisutch*) or masu salmon (*Oncorhynchus masou*) were deprived of food for 10 and 20 days, SH was significantly reduced (Sakai 1983). As the susceptibility of fish to disease agents increases under stressful conditions (Angelidis et al. 1987; Maule et al. 1989), it is possible that complement might be affected under such conditions.

After 16 days of intermittent disturbance, ACH_{50} and SH_{50} titers were significantly lower than control levels. Within the same period of time, CCP titers dropped markedly with respect to controls. In contrast, C3 levels

Fig. 1. Levels of plasma cortisol and glucose for the different experimental groups compared with pooled controls. No differences were found among controls sampled on different days. *, significantly different from the control value ($p < 0.05$).



showed a moderate but significant decrease after the same period of time. The levels of the three hemolytic activities varied in a similar pattern during the treatment. A significant correlation was found between SH_{50} and CH_{50} values in both control and stressed fish. Activities of SH and ACP were also correlated in a similar way. Nevertheless, ACP and CCP hemolytic activities were not correlated in control fish, but the correlation was highly significant in stressed fish. This correlation suggests that under normal conditions each pathway (ACP and CCP) follows a different pattern of activation. However, stress affects the two pathways in a similar fashion. Serum C3 levels fluctuated according to the hemolytic activities, suggesting that changes in the amount of C3 in serum produce consistent changes in the hemolytic activity. Although the changes observed in serum C3 might not be sufficient to mediate the changes in the different hemolytic activities, reduction of C3 in concert with a reduction of any other component of the complement activation cascade may have led to these results. Alternatively, the assay used for measuring C3 levels detects the C3 protein in serum regardless of its form (native (hemolytically active) or degraded (hemolytically inactive)). Hence, it is possible that part of the C3 detected in the assay could be hemolytically inactive.

The precise mechanism responsible for the reduction in complement levels is unknown, although it is very likely that corticosteroids are involved in this response. Corticosteroids have been associated with an immunosuppressive effect in fish (Schreck and Bradford 1990), and implantation of cortisol in fish has been reported to suppress certain immune responses (Thomas and Lewis 1987; Pickering and Pottinger 1989). Furthermore, cortisol has been reported to increase during the parr-to-smolt transformation of coho salmon (Maule et al. 1987) and, in this period, spontaneous hemolytic activity has been shown to decrease (Sakai 1983). A reduction in the serum bactericidal activity has been reported in rainbow trout (*Oncorhynchus mykiss*) during the spawning period (Iida et al. 1989), and circulating cortisol levels have also been shown to increase in fish during this period (Pickering and Christie 1981).

Sunyer and Tort (1995) previously showed that sea bream serum displays a strong hemagglutinating activity to RaRBC. This activity was not due to the presence of naturally occurring antibodies to RaRBC, as very little agglutination activity is lost after serum treatment with β-mercaptoethanol, which breaks immunoglobulins, resulting in a loss of agglutinating activity. In addition, this activity could be almost completely inhibited after incubation of serum with lipopolysaccharide. Furthermore, sea bream serum was able to agglutinate different bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, and *Micrococcus luteus*. Because the hemagglutinating activity displayed by sea bream serum could be removed after incubating the serum with lipopolysaccharide, a role for this agglutinating activity in the nonspecific immune response against bacteria is suggested. A significant reduction in hemagglutination was observed from the 3rd day of daily disturbance until the last day, when the activity decreased by 3 times compared with controls. Lectins have been reported to play a role in nonspecific defense mechanisms in fish, acting, for example, as opsonins (Alexander and Ingram 1992). Nevertheless, no previous studies have reported a down-regulation or down-modulation of these molecules under stressful conditions. In this study, the hemagglutinating activity of serum to RaRBC was significantly reduced in a short period of time. Hemagglutinins or lectins can therefore be included as additional parameters to monitor the effects of stress on the immune system of fish.

Part of the antibody population measured in our study likely consists of naturally occurring antibodies. These antibodies are normally present in fish serum (Gonzalez et al. 1988) and are characterized by their ability to combine with several kinds of antigens, although with rather poor affinity. These antibodies have also been reported to play a role in nonspecific defense mechanisms (Michel et al. 1990). Their titers were reduced to a very significant extent (57% of controls) after 16 days of repeated disturbance. Maule et al. (1989) and Mazur and Iwama (1993) demonstrated that stress and corticosteroids have suppressive effects on the humoral antibody production in immunized fish. In this study, we show that the production of antibodies in nonimmunized fish can also be affected by stress.

Circulating lymphocyte counts have been often used as a classical parameter to assess immunodepressive states in fish under perturbatory stimulus. Lymphocytopenia has been observed after a single acute stress (McLeay and Gordon 1977; Ellssaesser and Clem 1986), and also after 2 weeks of daily stress (Barton et al. 1987). In the present study, the same pattern was recorded, with the first lymphocytopenia reaching statistical significance after 3 days of treatment. The 7-day sample showed no significant difference, but a significant difference was again observed after 16 days. This last sample is in agreement with a more general immunodepressive status, as revealed by the humoral immune analysis. The lowered lymphocyte values could signal a depressed status in all samples compared with controls, except that variations in individuals make the intergroup differences significant. However, it should be noted that a fluctuating trend was apparent in most immune parameters in our work, with a first depression at 3 days, higher values at 7 days, and further depression at 16 days.

Plasma cortisol and glucose concentrations showed an expected increase in the response to stress. Cortisol levels increased at the first sampling of stressed fish (1 day) and continued to be significantly higher than the controls until day 16. It is well known that acute stress induces a sharp rise in plasma cortisol levels, and levels in some fish can increase by up to 100 times over basal values (Barton and Schreck 1987; Pickering et al. 1987). Basal cortisol levels recover from a single acute stress after some hours, depending on the intensity of the stressor (Pickering and Pottinger 1989; Vijayan and Moon 1994). In contrast, chronic stress also results in an increase in cortisol concentration but to a lesser degree, i.e., 2–3 times higher than basal levels (Pickering et al. 1987), and significantly higher levels of cortisol are recorded during several days and weeks following chronic treatments such as continuous confinement and crowding (Pottinger and Pickering 1992; Mazur and Iwama 1993). In the case of the daily acute stress applied in the present work, a significant fourfold rise in cortisol was observed after 1 day and sustained higher levels of cortisol were seen after 3, 7, and 16 days. The levels of cortisol after day 1 would indicate a response to the acute stress, although measured after 24 h. Previous work on sea bream subjected to short acute stress by capture showed a cortisol peak after 1 h, which declined thereafter (Molinero and Gonzalez 1995), so that values after 24 h could represent cortisol values following a recovery period. However, the occurrence of elevated cortisol levels on the following sample days indicates that fish did not adapt or habituate to the stress in the period analyzed. Studies in mammals showed that corticosteroid levels increase after each one of a repeated series of stressors, and decrease when the stressor disappears (De Boer et al. 1988). In fish, Barton et al. (1986) found a similar pattern, but the repeated stressors were applied at 3-h intervals. Unfortunately, few other studies have investigated the dynamics of glucose levels, cortisol levels, or other indicators after repeated stress. Rush and Umminger (1978) showed adaptation in the glycemic response after habituation to stress in goldfish (*Carassius auratus*), and Barton et al. (1987) showed habituation of the cortisol response after 10 weeks of daily stress in rainbow trout. Our results with sea bream show no habituation after 16 days. However, the possibility of habituation after longer exposures cannot be ruled out.

Glucose concentration was significantly increased 24 h after the first stress. This is a common response to acute stress and is driven by the release of catecholamines (Andersen et al. 1991). The hyperglycemia observed in our study was not severe, probably because of the 24 h that elapsed between the end of the stress and the sampling. (Unpublished results from our laboratory show typical control values in sea bream of about 50–70 mg/dL.) Glucose levels were similar thereafter, except for the last (16 day) sampling, when a significant decrease was observed. As fish were fed throughout the experiment, this decrease in the hyperglycemic response could not be due to fasting, although a reduction in food intake has been reported in sea bream after stress (Kentouri et al. 1994). Nevertheless, it is interesting to note that this decrease coincides with the depression of the indicators of the immune response.

In conclusion, daily application of an acute stress has clear immunodepressive effects, altering all the parameters we measured. The stressor applied was not considered to be severely harmful. A similar daily treatment over a longer period of time did not affect growth of rainbow trout (Barton et al. 1987). However, in our study the immunosuppressive effects lasted for at least 16 days, such that the risk for disease was increased during this period. Thus, aquaculture operations and other aquarium facilities should avoid repeated manipulation, which could have effects similar to those shown in this study. We found that the complement system was depressed under such conditions. This effect of stress has not been reported previously. Thus, processes involving the complement system in fish, such as bactericidal activity, viral inactivation, or opsonization, become depressed and may compromise fish survivorship. Therefore, we recommend complement titrations (either activities or specific components) to assess immunosuppression caused by stressful conditions, in support of the statement of Sakai (1992) who suggested including complement titrations in the conventional battery of tests used to assess the overall health status of fish.

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Estimating mean age at sexual maturity in the crabeater seal (*Lobodon carcinophagus*)

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Abstract: Mean age at sexual maturity (ASM) in crabeater seals (*Lobodon carcinophagus*) was estimated according to two different methods. Samples were from 1964, 1977, and 1989. Using the first method, the estimated ASM in 1989 was 6.57 ± 0.82 years, which was considerably older than reported earlier. This estimate is the only one existing for crabeater seals that can be used to compare populations and species. The first method was based on the maturity rate at age. Behavioural segregation of mature and immature seals could give skewed maturity rates in samples. Because the 1964 and the 1977 samples were collected during a season when segregated behaviour was expected (breeding season), results from these years were not comparable with the 1989 estimate (collected during moult). The second method does not produce absolute values of ASM, but the estimates could be used as indices of ASM within a specific population. There was a statistically significant increase in the ASM index in 1989 (4.93 ± 0.57), compared with 1964 (3.75 ± 0.31) and 1977 (3.80 ± 0.44). This is a strong indication of a true increase in ASM in crabeater seals. Recommendations are made to improve future procedures for studying reproductive status in seals.

Résumé : Nous avons estimé l'âge moyen à la maturité sexuelle (AMS) chez le phoque crabier (*Lobodon carcinophagus*) par deux méthodes différentes, à partir d'échantillons recueillis en 1964, 1977 et 1989. Selon la première méthode, l'AMS de 1989 était de 6,57 ± 0,82 ans, âge considérablement plus élevé que ce qui avait été signalé auparavant. C'est la seule estimation pour le phoque crabier qui puisse servir à comparer les populations et les espèces. La première méthode se fondait sur le taux de maturité selon l'âge. La ségrégation comportementale qui sépare les phoques matures et immatures a pu causer l'asymétrie des taux de maturité dans les échantillons. Étant donné que les échantillons de 1964 et 1977 ont été prélevés à une saison où la ségrégation était prévisible (période de reproduction), les résultats obtenus pour ces années-là n'étaient pas comparables aux estimations de 1989 (prélèvement pendant la mue). La deuxième méthode ne donne pas de valeurs absolues de l'AMS, mais les estimations pourraient servir d'indices de l'AMS dans une population donnée. L'augmentation de l'indice-AMS était statistiquement significative en 1989 (4,93 ± 0,57) par rapport à 1964 (3,75 ± 0,31) et à 1977 (3,80 ± 0,44). Il y a là un solide indice d'une croissance réelle de l'AMS chez les phoques crabiers. Nous faisons des recommandations pour améliorer les méthodes d'étude de l'état de maturité sexuelle chez les phoques.

[Traduit par la Rédaction]

Introduction

Age at sexual maturity (ASM) is one of the vital parameters in population dynamics, and has been calculated for most species of pinnipeds (for a summary see Riedman 1990). Variations in ASM among years have been used as indicators of long-term changes in nutritional status of populations (on the basis of the correlation between ASM and body growth; Laws 1956). Changes in ASM have also been correlated with changes in population density, as in harp seals (*Phagophilus groenlandicus*) (Bowen et al. 1981), and with changes in food competition, e.g., crabeater seals (*Lobodon carcinophagus*) (Bengtson and Laws 1985).

However, before conclusions at the level of populations and ecosystems are made, factors affecting estimates of ASM must be studied in detail. The choice of statistical method for calculating mean ASM can be crucial, since different methods have been shown to produce different results from the same artificial set of data (DeMaster 1984). Furthermore, methods of classifying maturity status must be well defined (York 1983). Also, inconsistent sampling in time or space can introduce serious biases (juvenile and mature animals often differ in behaviour, which could affect their likelihood of getting sampled) (McLaren and Smith 1985).

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A common method of estimating ASM is based on the reproductive status at the time of collection (DeMaster 1978; York 1983). A contrasting method, which relies on signs of past reproductive performance, i.e., number of ovarian corpora, has been used to calculate mean ASMs in female crabeater seals (Øritsland 1970; Bengtson and Siniff 1981). A trend to increasing mean ASM in crabeater seals through the 1960s and 1970s was suggested by Bengtson and Laws (1985). However, this result must be treated with caution, since information on sample sizes, time of sampling, and variance of means for the samples is lacking.

The aim of this study is to evaluate if changes in mean ASM have occurred in recent samples of crabeater seals and to discuss influences of different sources of error on ASM estimates.

Materials and methods

Reproductive tracts of 47 female crabeater seals were collected during a Swedish Antarctic expedition (the Stena Expedition) between 23 February and 12 March 1989, at the Erebus and Terror Gulf (63°47′S, 56°27′W), Wilhelmina Bay (64°39′S, 62°08′W), and Bigourdan Fiord (67°31′S, 67°10′W). Additional data on 30 females were obtained from the U.S. Surveyor Expedition (9–13 January 1989), which were pooled with the Stena data in the calculations. Furthermore, published data from two earlier samples collected on 1–14 November 1977 (Bengtson and Siniff 1981), and 25 August – 22 September 1964 (Øritsland 1970), were recalculated and compared with the recent data. Complementary information (on the number of juvenile animals) for the 1964 sample was provided by Havforskningsinstituttet, Bergen, Norway. All samples were taken in the region between the South Shetland Islands and the Antarctic Peninsula.

Tracts were detached immediately below the cervix, and uterine horns were opened from the uterotubal junction to the cervix, before fixation in 10% buffered Formalin. The age of the females was determined by counting annual cementum layers in longitudinal sections of postcanine teeth (Bengtson and Siniff 1981). All ovaries were weighed and sliced serially (about 2 mm thick) using a razor blade, whereafter the slices were inspected in sequence. The largest follicle, corpus luteum (CL), and corpora albicantia (CAs) were measured along three axes. Two perpendicular measurements were made in the section where they reached their maximum diameter, and the third axis was estimated by counting the number of slices containing structures of the corpus or follicle. The total numbers of CAs and follicles exceeding 2 mm in diameter were counted.

Statistical methods

All analyses were based on the definition that ASM is the age of an individual female at first ovulation (York 1983).

Calculation of ASM by method 1

In this method the proportion of mature females in each age-class is used to calculate mean age at first ovulation and corresponding 95% confidence intervals (DeMaster 1978). Because the females from 1989 were collected well after the breeding season, all mature and fertile females were expected to have ovulated. Therefore, the presence of a CL was used as a criterion of maturity. The sample from 1977 was collected during the mating period, when only some females could be assumed to have ovulated. In this sample, the presence of large follicles (≥10 mm) was used as a complementary indicator of sexual maturity (Bengtson and Siniff 1981). Since natural variation or sampling error can produce negative probabilities of ovulating in some age-classes, we also fitted observed data to several functions. The Gompertz function, $f(x) = \exp(-b\exp(-kx))$, was used because it produced the least residual sums of squares. Age-specific values from this fitted curve were then treated in the same way as the observed data.

Calculation of ASM by method 2

This method (Bentgson and Siniff 1981) is based on the following assumptions. Female crabeater seals ovulate once each year in alternating ovaries. The erupted follicle forms a CL that is converted into a CA after gestation. The CA persists, and can be distinguished from other structures at least 5 years after formation. To obtain the age of a female at her first ovulation, the total number of ovarian corpora is subtracted from her age. Females that could have ovulated for the first time more than 5 years prior to the sampling are not included (i.e., no female more than 5 years older than the youngest maturing age-class in a specific sample is included). The mean age at first ovulation is calculated as the arithmetic mean of these back-calculated ages. In the present study, the mean ASM is given with 95% confidence interval (CI) (Student's $t_{0.05}$, df = $n - 1$).

Results

The presence of mature animals in different age-classes varied considerably among years. In the 1964 sample, two of three females reached maturity during their 4th year, and all younger animals were juvenile and all older animals were mature (Table 1). In the 1977 sample, the youngest mature female was 2 years old, and all females aged 4 years and older had already reached maturity (Table 2). The more recent 1989 sample was even more divergent; the youngest mature female was found in age-class 4, and some of the females did not ovulate for the first time until age 9 (Table 3).

Method 1

We calculated mean ASM for each sample with method 1. In 1964 the mean ASM was 4.33 years (95% CI, 3.68–4.98), which was not significantly different from the 1977 sample (3.64 years; 95% CI, 3.25–4.03). For the 1989 sample, ASM was estimated at 6.57 years (95% CI, 5.75–7.39), which was significantly higher than in both earlier samples (1964 and 1977) (Tables 1, 2, and 3). Using fitted values of the Gompertz function the analysis produced only marginally higher estimates and variances, which consequently did not change the main results (Table 4).

Method 2

Because the youngest maturing age-class differed among samples, the ages of females that could be incorporated using method 2 varied as well. Therefore, 24 mature females

Table 1. Age at first ovulation in the 1964 sample (data recalculated from Øritsland (1970)) according to the method of DeMaster (1978).

Age (x)	$n(x)$	$t(x)$
1	5	0
2	5	0
3	8	0
4	3	2
5	5	5
6	7	7
7	4	4

Note: Mean age at first ovulation is 4.33 ± 0.65 years (±95% confidence interval). Calculation of mean age at first ovulation is explained in Table 3. $n(x)$, total number of females at age x; $t(x)$, number of mature females.

Table 2. Age at first ovulation in the 1977 sample (data recalculated from Bengtson and Siniff (1981)) according to the method of DeMaster (1978).

Age (x)	$n(x)$	$t(x)$
1	10	0
2	14	1
3	7	2
4	8	8
5	3	3
6	2	2
7	4	4

Note: Mean age at first ovulation is 3.64 ± 0.39 years (±95% confidence interval). Calculation of mean age at first ovulation is explained in Table 3. $n(x)$, total number of females at age x; $t(x)$, number of mature females.

between 4 and 9 years old could be incorporated in the 1964 sample, 20 females between 2 and 7 years old, in 1977, and 14 females 4 to 9 years old in the 1989 sample. Age at first ovulation for each of these females was estimated and used to calculate the mean ASM of the samples. A pattern similar to that for method 1 emerged. There was no significant difference between the two earliest samples (3.75 years in 1964 (95% CI, 3.44–4.06), 3.80 years in 1977 (95% CI, 3.36–4.24), while the estimated ASM in 1989 was significantly higher (4.93 years; 95% CI, 4.35–5.51) (Table 5).

The age structure of the three samples varied considerably (Tables 1, 2, and 3, column $n(x)$). Therefore, the robustness of method 2 for such variations was tested. Simulated age structures were applied to the existing proportion of first-time ovulators in each age-class. It was found that the method was robust against such changes. The estimates of mean ASM did not change more than ±0.13 when simulated changes in mortality rates were varied by ±20%.

Table 3. Age at first ovulation in the 1989 sample estimated according to DeMaster (1978).

Age (x)	$n(x)$	$t(x)$	$f(x)$	$P(x)$	$xP(x)$	$v(x)$
1	5	0	0			
2	2	0	0	0	0	0
3	8	0	0	0	0	0
4	11	1	0.09	0.09	0.36	0.01
5	4	0	0	−0.09	−0.46	0
6	8	7	0.88	0.88	5.25	0.02
7	3	2	0.67	−0.21	−1.46	0.11
8	5	4	0.8	0.13	1.07	0.04
9	6	6	1	0.2	1.80	0

Note: Mean age at first ovulation is 6.57 ± 0.82 years (±95% confidence inverval). $n(x)$, total number of females at age x; $t(x)$, number of mature females; $f(x)$, proportion of mature females; $P(x)$, probability of ovulating for the first time at each age, i.e., $(f(x) - f(x-1))$; $xP(x)$, age multiplied by $P(x)$, the sum of this column giving the mean value; $v(x)$, variances for each year $(\sum f(x)\cdot(1 - f(x))/(n(x) - 1) + w^2\cdot f(w)\cdot(1 - f(w))/(n(w) - 1))$, the sum of this column giving the total variance; w, maximum age in sample.

Table 4. Summary of estimates of age at maturity in the crabeater seal during 3 years according to the method of DeMaster (1978).

Sample	DeMaster (1978)	DeMaster (1978) Gompertz	Gompertz parameters b	Gompertz parameters k
1964	4.33±0.65	4.41±0.86	30.02	1.02
1977	3.64±0.39	3.78±0.68	30.01	1.21
1989	6.57±0.82	6.61±1.01	30.02	0.65

Note: Negative probabilities of ovulating for the first time were produced in some years (as in Table 3, column $P(x)$). To check how much these irrational values influenced the ASM estimate, sampling data were fitted to the Gompertz function: $f(x) = \exp(-b\exp(-kx))$, where $f(x)$ is the proportion mature in each age class, x is the the age-class, and b and k are constants estimated by iterative methods (SYSTAT).

Data for a critical test of the assumptions made in method 2 were provided by the number of young females with one versus two corpora. If the ovulation pattern was as assumed, the expected number of females with one corpus should have been slightly higher than the number with two, since some females might have died between the first and second ovulations. In our results the ratio was 1:6, and in the results of Bertram (1940) it was 1:9. When these samples were combined, a ratio of 2:15 resulted, which was significantly different from the expected ratio (9:8) (chi-square test, $P < 0.001$). Thus, the assumption of a single ovulation in the year of puberty appeared to be invalid.

Comparison of methods

A comparison of the results obtained by the two methods for the same samples showed no significant difference between the methods in 1964 or 1977, whereas for the

Table 5. Number of females ovulating for the first time at age x in three different samples.

	Sample		
Age (x)	1964[a]	1977[b]	1989[c]
1	0	0	0
2	0	2	0
3	9	4	2
4	13	11	1
5	1	2	7
6	1	1	4
7	0	0	0
Sum	24	20	14
Mean	3.75	3.80	4.93
95% CI	0.31	0.44	0.57

Note: The age at first ovulation is back-calculated from ovarian corpora according to the method by Bengtson and Siniff (1981). Mean age at first ovulation is given with 95% confidence interval (CI).

[a]Data from Øritsland (1970).

[b]Data from Bengtson and Siniff (1981).

[c]Present study.

1989 sample, method 1 produced a significantly higher estimate than method 2 (Tables 4 and 5).

Discussion

Critique of the methods

The two main results are as follows. First, a statistically significant increase in ASM was detected for the 1989 sample compared with the two earlier ones by both methods of calculation. Second, the two methods produced similar mean ASMs for the samples from 1964 and 1977, but the estimates differed for the 1989 sample.

We think the main explanation for the finding that the two methods produced similar estimates of ASM for two samples but not the third is their reliance on two different factors. The method of DeMaster (1978) (method 1) is sensitive to the extent to which sampled proportions of immature and mature animals are representative of the population (see below), whereas the method of Bengtson and Siniff (1981) (method 2) depends on the assessment of the reproductive history of each female.

Method 1 does not involve the potential errors of estimating ASM from the reproductive history of individual specimens, since the maturity rates of each age-class on the day of collection are used. However, these maturity rates must be representative of the population (McLaren and Smith 1985). A bias is introduced if there is a difference in the probability of sampling mature and immature animals. Such a difference should logically be most pronounced during the breeding season, when more mature animals are on the ice (Siniff et al. 1979). Another source of bias in this method is that all age-classes are evenly weighted, although the younger classes comprise larger proportions of the population. This gives the result a slightly positive bias (DeMaster 1984).

In method 1 the ratio of mature animals should increase with age; otherwise, negative probabilities of ovulating in some age-classes are produced (see Table 3, column $P(x)$). To check how much such irrational values influenced the ASM estimate, sampling data were smoothed. In our case, the main results did not change when smoothed data were used (Table 4).

Method 2 is based on back-calculation of ovarian scars and relies on two assumptions: crabeater seals have a regular pattern of one yearly ovulation, and the resulting CA persists 5 years. If these assumptions are valid, the number of females with one ovarian corpus is expected to exceed the number of females possessing two corpora. Bertram (1940) noted an apparent underrepresentation of females with one corpus versus females with two (ratio 1:9). Our corresponding data (1:6) pooled with the data from Bertram (1940) are significantly different from the expected ratio. It is also noteworthy that one of the two primiparous females in this study had two corpora and thus had experienced an unsuccessful ovulation. Double ovulations in nulliparous females are also known among other marine mammals (e.g., Read 1990). If crabeater females often ovulate twice during their 1st year of puberty, then the first assumption of method 2 would not be valid and the estimate of ASM would be too low.

The second assumption (on the regression rate of CAs) is even more uncertain. Even though findings of earlier studies were that CAs persist at least 5 years in crabeater seals (Øritsland 1970; Bengtson and Siniff 1981), there are problems with small CAs. They may be missed (biasing ASM upwards) or confused with other similar structures such as atretic follicles (leading to a downward bias) (Boyd 1984).

Estimates of the mean ASMs did not differ significantly between methods for the 1964 and 1977 samples. If double ovulations decreased the estimate using method 2 (as discussed above), then the estimate using method 1 would also have been too low, but for a different reason. Both the 1964 and the 1977 samples were collected during the breeding season when overrepresentation of the mature females from each age-class could be expected, which might have explained the low estimate obtained by method 1.

The 1989 sample was collected during moult, a period when all year-classes were more likely to be represented as assumed in method 1 (since all seals have to spend much time out of the water to facilitate fur shedding). The mean ASM of method 1 was more than 1.5 years higher than the estimate of method 2 for the same sample. If double ovulations in nulliparous females were common in crabeater seals, this could have explained the lower estimate of method 2 in this year as well. The fact that method 1 is slightly positively biased, together with the influence in the same direction of the two aberrant females (juveniles at 7 and 8 years of age) in this sample, may explain the difference observed.

The difference in ASMs of samples should not have been caused by differences in age structures. The age structures of the samples varied, in part because of small sample sizes, but most likely also because of fluctuations in cohort strength (Testa et al. 1991). However, the mean

values obtained by method 2 proved to be robust to simulated changes in age structures, and estimates of the mean ASM obtained by method 1 were not dependent on changes in age structure as long as the proportion of mature animals in each age-class remained constant. Neither should the difference in ASMs of the samples have been caused by differences in the examination procedure for CAs, since efforts were made to examine the CAs with the same technique in all samples.

Conclusions on use of methods

Samples from 2 years (1964 and 1977) showed similar estimates of ASM using the method of DeMaster (1978) (method 1) (4.33 ± 0.65 and 3.64 ± 0.39). Collections were made during the breeding period, when mature animals were likely to be overrepresented in samples. We therefore suggest that the ASM estimates for these two samples were lower than the true values for the whole population. The sample from 1989 was collected during moult (when samples are expected to be more representative of the population). In this sample the estimated ASM was 6.57 ± 0.82 years, which was considerably older than earlier reported in crabeaters. Unfortunately, results for the two earlier samples could not be directly compared with the 1989 sample using method 1, since they were collected during breeding seasons. Consequently, the estimate based on the 1989 sample (using method 1) is the only one available on ASM in crabeater seals that can be used in studies comparing populations and species.

The method of Bengtson and Siniff (1978) (method 2) suffers from its dependency on unverified assumptions of ovulation patterns and regression rates of ovarian corpora in crabeater seals. As long as these factors remain unknown, estimates calculated by this method cannot be used as absolute values. Therefore, estimates of ASM obtained by method 2 should not be used in comparisons among species.

The advantage of method 2 is that it is not explicitly sensitive to sampling time (since it is based on mature females only). Provided that small ovarian corpora are determined in an identical manner for all samples, we suggest that estimates obtained by method 2 can be used as indices of ASM within a specific population. According to results achieved in this study, there is a statistically significant increase in the ASM index in 1989 (4.93 ± 0.57), compared with 1964 (3.75 ± 0.31) and 1977 (3.80 ± 0.44). This is a strong indication of a true increase in ASM in crabeater seals. Additional data from samples representative of the population are needed to evaluate the magnitude of changes in ASM.

Recommendations for collection and treatment of reproductive data

(*i*) The time of sampling should be chosen in such a way that periods of segregated haul-out behaviour of mature and immature animals are avoided. For most species of seals this means that collection should be done preferably during moult. It could prove important to sample over the whole span of the moulting period, since there is evidence that some fractions of a population moult earlier than others (e.g., in harbour seals, *Phoca vitulina* (Thompson and Rothery 1987)). Another advantage of sampling well after the breeding season is that all females that are going to ovulate during that season have done so, and developed one (or two) clearly visible CL.

(*ii*) For comparative studies of ASM among different species or for trend analysis within a population, the method of DeMaster (1978) is recommended, provided that sampling is conducted as described above. DeMaster's method (1978) should be complemented with a curve fit of raw data, especially in cases where maturity ratios do not increase successively with age.

(*iii*) It could be important to routinely analyse a sample by more than one method, since different methods have different weak points. A method such as that of Bengtson and Siniff (1981) (preferably with more strictly defined rules of classification) can be used to obtain an index of reproductive status. Obviously, such indices are only useful within a specified population.

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The nonrandom size distribution and size-selective transport of age-0 Atlantic tomcod (*Microgadus tomcod*) in the lower Hudson River estuary

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Abstract: From early March until about mid-May 1975 and 1976, the entire Hudson River population of recently hatched Atlantic tomcod, *Microgadus tomcod*, was sorted, by estuarine hydrodynamic forces, into a distinct longitudinal size gradient throughout a 93-km section of the lower estuary. This distribution was evident as a well-ordered progression of mean lengths, beginning upriver with the smallest larvae and increasing in a downriver direction, with the largest larvae being nearer the estuary mouth. Aspects of the passive estuarine transport of larval tomcod during March and April, and the active upriver migrational movement of juveniles greater than 20 mm in length beginning in late April, were deduced from systematic changes in the longitudinal distribution of tomcod mean lengths and associated variances. Abrupt increases in tomcod mean length, variation in length, and population density often occurred just seaward of the 1.0‰ salinity-intrusion boundary. During March–May, vulnerability of tomcod to power-plant entrainment at km 60 – km 69 increased whenever freshwater flow decreased and the 1.0‰ intrusion boundary moved upriver. The late April – early May appearance each year of age-0 tomcod in impingement samples at km 69 is consistent with the hypothesis of an upriver migration of juvenile tomcod beginning in late April.

Résumé : Du début mars à environ la mi-mai, en 1975 et 1976, toute la population de poulamon atlantique, *Microgadus tomcod,* nouvellement éclos de l'Hudson s'est trouvée triée, du fait des forces hydrodynamiques de l'estuaire, selon un gradient longitudinal particulier de tailles tout au long d'un tronçon de 93 km du bas estuaire. Cette distribution apparaissait sous la forme d'une progression bien ordonnée des longueurs moyennes, qui commençait en amont avec les larves les plus petites et qui augmentait vers l'aval, les larves les plus grosses se trouvant près de l'embouchure de l'estuaire. On a pu déduire certains aspects du transport passif dans l'estuaire des larves de poulamon en mars et avril, et du mouvement actif d'amontaison des juvéniles de plus de 20 mm à partir de la fin d'avril, d'après les changements systématiques de la distribution longitudinale des longueurs moyennes des poulamons et des variances associées. Les augmentations brutales de la longueur moyenne des poissons, des variations de la longueur et de la densité de la population se produisaient souvent juste en aval de la limite de la marée saline à 1‰. De mars à mai, la vulnérabilité des poulamons à l'entraînement par la centrale électrique aux km 60–69 augmentait dès que le débit d'eau douce baissait et que la marée saline à 1‰ remontait le fleuve. L'apparition annuelle à la fin d'avril et au début de mai, de poulamons d'âge 0 dans les échantillons prélevés au km 69 concorde avec l'hypothèse d'une amontaison des jeunes poulamons à partir de la fin d'avril.

[Traduit par la Rédaction]

Introduction

At the insistence of the Nuclear Regulatory Commission and the U.S. Environmental Protection Agency, and as part of a 1981 legal settlement, the Atlantic tomcod, *Microgadus tomcod*, was one of three major fish species whose long-term population dynamics were to be defined in relation to large-scale operations by the Hudson River electric utilities (Barnthouse et al. 1988). However, the 1975–1990 design used by the utilities for their March–May ichthyoplankton sampling, as described by Klauda et al. (1988) and other utility consultants (e.g., Lawler, Matusky, and Skelly Engineers 1991), did not include the region between km 0 at the estuary mouth and km 22; this region represents nearly one third of the estuary downriver from the Indian Point Nuclear Generating Station (km 69). The use of this design has impeded meaningful assessment of the ecology and population dynamics of larval and early juvenile

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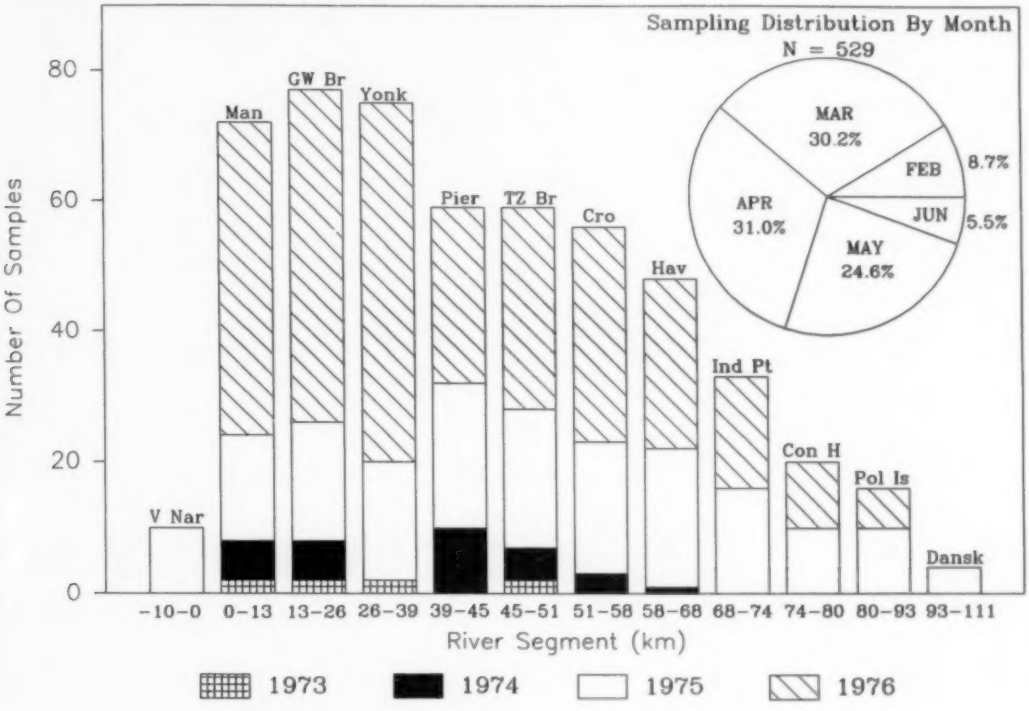
Fig. 1. Map of the Hudson River estuary showing the 1973–1976 downriver sampling boundary beyond the estuary mouth at the Verrazzano Narrows Bridge (km –10), the estuary mouth at the Manhattan Battery (km 0), and the upriver sampling boundary at Danskammer North (km 111). (From Dew and Hecht 1994*b*. Reprinted with permission.)

Atlantic tomcod in the Hudson River. In consequence, little is known of the passive transport, active migration, or spatial distribution of larval and early juvenile tomcod within the estuary. Such knowledge is fundamental to any useful evaluation of the habitat of age-0 tomcod, their availability as prey for other fish species, and their vulnerability to entrainment and impingement at cooling-water intakes along the Hudson.

The Atlantic tomcod is the only abundant winter-spawning fish species in the Hudson River. The young-hatch in the river's limnetic zone during February and March (Dew and Hecht 1994*a*), when the amount and variability of freshwater flow is approaching an annual maximum (Mancroni et al. 1992; Wells and Young 1992). Demersal tomcod eggs, because they do not move far from where they are spawned (km 80 – km 130), remain in a relatively stable, mostly freshwater environment for 60–65 days until hatching (Dew and Hecht 1994*a*). In contrast, the newly hatched larvae are quickly swept by high freshwater flow downriver to the most seaward reaches of the estuary. Here, in waters of 2–15‰ salinity during March–May, they undergo large-scale, saltatory, bidirectional movements between km 0 and km 69, in association with the flow-driven movements of the 1.0‰ salinity-intrusion front (Dew and Hecht 1994*a*).

Despite the magnitude and the random, largely unpredictable variation of the hydrodynamic regime during spring runoff, the Hudson River estuarine transport process imposes

Fig. 2. Spatial and temporal allocation of ichthyoplankton sampling effort for Atlantic tomcod in the Hudson River estuary during 1973–1976. Because the sampling distribution generally followed the tomcod distribution, the allocation of sampling effort among regions was unequal. Abbreviations follow the names in Fig. 1. (From Dew and Hecht 1994*b*. Reprinted with permission.)



an unexpected degree of nonrandom order on the longitudinal distribution of Atlantic tomcod. In this paper I describe the stability and duration of this order and hypothesize it to be the result of interaction between tomcod behavior and estuarine, two-layer flow. Also, I describe a reversal in the river-long gradients of tomcod length and variance as the fish grew from a passive-transport phase into an active, upriver-migration phase.

Materials and methods

The sampling area of this 2-year (1975 and 1976) study included the 121-km stretch of the lower Hudson River between km −10 (Verrazzano Narrows) and km 111, where km 0 is the estuary mouth at the Manhattan Battery and the Statue of Liberty (Fig. 1). All sampling was conducted in the main channel of the estuary where the depth ranged from 10 m at the George Washington Bridge station (km 18) to 26 m at the Con Hook station (km 80). A total of 490 ichthyoplankton tows were made at 19 stations in 1975 and 1976 (Dew 1991). Because the sampling distribution generally followed the tomcod distribution, the allocation of sampling effort among stations was unequal (Fig. 2). The design was based on data from preliminary sampling in April 1973 and April–May 1974 (Dew 1991). In 1975 and 1976, weekly sampling was conducted from mid-February to mid-May (1975) or mid-June (1976). Temperature and salinity were measured at sample depths after each tow was completed. Physical data, tomcod abundance, and mean length of the tomcod collected in each sample are in Dew (1991, Appendix 1).

Operative flow (Q_f; cubic metres per second) on a given sampling date was estimated from the kilometre location of the 1.0‰ salt front (SF) as

$$Q_f = 2957.2e^{-0.045(SF/1.609)}$$

modified from Dew and Hecht (1994*a*). Larval total length (TL) was measured to the nearest 0.1 mm, and post-yolk-sac larvae (post-larvae) were defined as juveniles after 15 April. All tomcod in samples of less than 100–150 fish were measured except where damage prevented accurate measurements. Samples with more than 100–150 tomcod were subsampled to obtain a random sample of 100–200 measurements.

All tows were made in daytime with 6.1 m long, non-closing, 1 m diameter, cylinder-cone Henson nets of 571-μm mesh, designed according to Tranter and Smith (1968) to optimize filtration efficiency. Nets were towed at discrete depths at 1.6–1.7 knots and hauled back by hand, thus minimizing the potential for sample contamination from other depth strata (which would occur if the nets were winched back under tow). Large floats were attached to the nets at the bridle-tow line juncture, with just enough float line to permit the nets to fish at the desired depth

(e.g., about 1 m from the bottom for bottom tows). The bottom and midwater nets were rigged at the bridle – tow line juncture with a Scripps depressor (Isaacs 1953, cited by Gehringer and Aron 1968) that, under tow, extended 0.6–0.8 m below the net ring. Each net was equipped with a calibrated, center-mounted TSK flow meter, and the average volume filtered (± 95% confidence interval) for 487 5-min tows in 1975 and 1976 was 202.1 ± 4.6 m^3. Comparison of the length distributions of age-0 tomcod collected by the metre net and a 9.1-m otter trawl indicated that metre-net avoidance by Atlantic tomcod did not cause significant bias during this study (Dew and Hecht 1994*b*).

The allocation of sampling effort to depth strata changed between and within years as the accumulated data more closely defined seasonal changes in the depth distribution of tomcod larvae. In 1975, surface and bottom tows were conducted from 22 February to 20 April, when surface tows were discontinued in favor of replicate bottom tows. Only trace concentrations of tomcod were present in surface tows after 23 March 1975. The 1976 program consisted of surface and bottom tows from 14 February through 14 March, mid-depth and bottom tows from 21 March through 9 May, and (because no tomcod larvae were collected in mid-depth tows on 9 May) only bottom tows from 15 May through 13 June. The changing distribution of effort reflected the changing distribution of the fish, and the decision to discontinue sampling within a depth stratum was based on the observation that there were no longer substantive numbers of larvae within that stratum. Of all (48 709) age-0 tomcod collected in 1975 and 1976, 1.5% were from the surface stratum, 14.8% were from the mid-depth stratum, and 83.7% were from the bottom stratum. The methods used to obtain standing-crop and population-density estimates are in Dew (1991, Appendices 1–4) and in Dew and Hecht (1994*b*).

After hatching was complete, the Hudson River population of Atlantic tomcod larvae and early juveniles was considered to be closed, i.e., the population size decreased because of mortality but did not change via emigration or immigration. It was also assumed, on the basis of data collected from km −10 to km 111, that the entire larval and early juvenile populations present in 1975 and 1976 were located within the river region between km 0 and km 93 from mid-February to June. Detailed information consistent with these assumptions can be found in Dew and Hecht (1994*a*).

The variance in mean length of age-0 tomcod was modelled as a response surface shaped by time and space. Observed within the context of stable length gradients, systematic changes in the river-long distribution of variance were used to make inferences about fish movement. Using the methodology of Box and Draper (1987), variance (Y) was modelled as a quadratic surface, in two variables X_1 and X_2, as

$$\ln (Y + 1) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2 + \beta_5 X_1 X_2$$

This model, linear in β and quadratic in X, relates the log-transformed variance (ln (Y + 1)) in mean tomcod length to time (X_1 is days from the end of February) and space (X_2 is the river kilometre from the Manhattan Battery).

Using matrix notation, estimates for the β parameters were calculated via Mathematica 2.2.1 (Wolfram 1991) as

$$\mathbf{b} = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{Y}$$

where $\mathbf{b}$ is the ($p \times 1$) vector of parameter estimates, $\mathbf{Y}$ is the ($n \times 1$) vector of log-transformed variance (+1) estimates, $\mathbf{X}$ is the ($n \times p$) matrix of independent variables, $\mathbf{X}'$ is the ($p \times n$) transpose of $\mathbf{X}$, p is the number of β parameters to be estimated, and n is the number of paired observations (X_1, X_2) (Draper and Smith 1966). The back-transformed variance for each set of paired observations was obtained as

$$Y = e^{(b_0 + b_1 X_1 + b_2 X_2 + b_3 X_1^2 + b_4 X_2^2 + b_5 X_1 X_2)} - 1$$

As an example, I used the data for March 1976 to obtain the vector of estimates ($\mathbf{b}_i$) for parameters (β_i):

$$\begin{array}{l} \ln (Y+1) \\ \\ \begin{bmatrix} 0.5504 \\ 0.2654 \\ 0.2151 \\ 0.1476 \\ 0.1432 \\ 0.1169 \\ 0.6544 \\ 0.5134 \\ 0.5110 \\ 0.2367 \\ 0.1432 \\ 1.3064 \\ 0.9509 \\ 0.5417 \\ 0.4725 \\ 0.3038 \\ 1.0956 \\ 0.8821 \\ 0.8574 \\ 0.7021 \\ 0.6544 \end{bmatrix} \end{array} \quad \boldsymbol{Y} = \quad \begin{array}{c} X_0 \quad X_1 \quad X_2 \quad X_1^2 \quad X_2^2 \quad X_1 \cdot X_2 \\ \\ \begin{bmatrix} 1 & 7 & 10 & 49 & 100 & 70 \\ 1 & 7 & 23 & 49 & 529 & 161 \\ 1 & 7 & 29 & 49 & 841 & 203 \\ 1 & 7 & 39 & 49 & 1521 & 273 \\ 1 & 7 & 58 & 49 & 3364 & 406 \\ 1 & 7 & 84 & 49 & 7056 & 588 \\ 1 & 14 & 32 & 196 & 1024 & 448 \\ 1 & 14 & 55 & 196 & 3025 & 770 \\ 1 & 14 & 61 & 196 & 3721 & 854 \\ 1 & 14 & 68 & 196 & 4624 & 952 \\ 1 & 14 & 87 & 196 & 7569 & 1218 \\ 1 & 21 & 3 & 441 & 9 & 63 \\ 1 & 21 & 16 & 441 & 256 & 336 \\ 1 & 21 & 22 & 441 & 484 & 462 \\ 1 & 21 & 35 & 441 & 1225 & 735 \\ 1 & 21 & 48 & 441 & 2304 & 1008 \\ 1 & 28 & 16 & 784 & 256 & 448 \\ 1 & 28 & 29 & 784 & 841 & 812 \\ 1 & 28 & 32 & 784 & 1024 & 896 \\ 1 & 28 & 39 & 784 & 1521 & 1092 \\ 1 & 28 & 52 & 784 & 2704 & 1456 \end{bmatrix} \end{array} \quad \boldsymbol{X} =$$

$$\boldsymbol{b} = (\boldsymbol{X}'\boldsymbol{X})^{-1}\boldsymbol{X}'\boldsymbol{Y} = \begin{bmatrix} 0.2826630 \\ 0.0553865 \\ -0.0133863 \\ -0.0005033 \\ 0.0001172 \\ -0.0003517 \end{bmatrix} \begin{matrix} b_0 \\ b_1 \\ b_2 \\ b_3 \\ b_4 \\ b_5 \end{matrix}$$

Growth was calculated separately for upriver (km 32 – km 93) and downriver (km 0 – km 31) tomcod as

$$\ln (L_t) = \ln (L_0) + G t_L$$

where t_L is the age of fish of length L in days after t_0, L_0 is the total length (millimetres) at time t_0, L_t is the total length (millimetres) at time t, and G is the instantaneous growth-rate coefficient. The quantities t_0 and t_L were calculated as

$$t_0 = \Sigma(Jd_i)/\Sigma d_i$$

and

$$t_L = (\Sigma(Jd_i)/\Sigma d_i) - t_0$$

where J is a consecutive day of the year corresponding to a sampling date, d_7 is the density of newly hatched larvae less than 7.5 mm in length on day J, and d_i is the density of the length class of interest on day J.

Maintaining the separation between upriver and downriver regions, the density distribution of each 1.0 mm length cohort was plotted over time, from its first appearance to its disappearance from the population (Hackney and Webb 1978; DeAngelis et al. 1980). Within a region, the density-weighted mean date for the distribution of a given length cohort through time was the date (t_L) on which the fish of that region attained length L. For example, the mean length of a newly hatched larva in 1975 and 1976 was 7.0 mm (Dew and Hecht 1994*b*). Within each region the average density of all larvae less than 7.5 mm in length was calculated for each sampling date. Multiplying density (weighting factor) by sampling date (expressed as J), summing the products, and dividing this by the sum of the densities resulted in the weighted mean date upon which the average larva attained 7.0 mm. For the newly hatched 7-mm category (5.7–7.4 mm), this was also the average hatching date (t_0). This procedure was repeated for subsequent 1.0 mm length cohorts, separately for each region, to obtain the date (now expressed as $t_L - t_0$, or the number of days from day 0) on which larvae of that length cohort attained their size (the midpoint of the length interval). The regression coefficient of ln L_t plotted against t (larval age) was an estimate of the instantaneous growth rate (G) of individual larvae.

Results

Mean length

In the spring of 1975 and 1976, from early March until about mid-May, the entire Hudson River population of recently hatched, semiplanktonic Atlantic tomcod was sorted, apparently by estuarine hydrodynamic forces, into a distinct longitudinal size gradient throughout a 93-km length of the lower estuary. This systematic distribution was evident (Tables 1 and 2) as a well-ordered progression of sampled mean lengths, beginning upriver with the smallest larvae and increasing in a downriver direction to the largest larvae near the estuary mouth (km 0).

The gradient in larval length was present both during and after tomcod hatching. During hatching in early March, the size gradient was maintained by a stream of larvae hatched in the Indian Point – Con Hook region (km 68 – km 87) (Dew and Hecht 1994*a*) and moved downriver by freshwater flow, with the earliest hatched, oldest larvae in the vanguard. In this way the size gradient during peak hatching on 7 March 1976 (Table 2) was maintained despite high freshwater flow (~1439 $m^3 \cdot s^{-1}$).

Hatching was complete by mid-March, but the size gradient persisted well into May, demonstrating that recruitment was not necessary to maintain the gradient. However, the post-hatch gradient tended to be disrupted temporarily by high freshwater flow. For example, high flow on 4 April 1976 (~1439 $m^3 \cdot s^{-1}$) mixed the larvae into a homogeneous assortment of lengths (Table 2), while pushing the entire population into a relatively small sector of the estuary between the Statue of Liberty (km 0) and the Harlem River (km 22). This well-mixed state was remarkably short lived. During the next 5 d, as river flows decreased to about 467 $m^3 \cdot s^{-1}$ on 10 April, a well-defined size gradient was reestablished throughout the 51-km region from the George Washington Bridge (km 18) to the Indian Point Nuclear Generating Station (km 69) (Table 2).

Close examination of the length gradient on each sampling date showed a transition zone associated with the 1.0‰ salinity-intrusion boundary. Abrupt increases in tomcod mean length, variation in length, and population density often occurred just seaward of the 1.0‰ salt front, regardless of rapid, large-scale movements of the front caused by changes in freshwater flow (Fig. 3). For example, between 7 and 14 March 1976, low flow permitted the salt front to move upriver a distance of 46 km, from the Harlem River (km 22) to the vicinity of Indian Point (km 68). The movement of this water mass transported much of the larval population from the George Washington Bridge (km 18) upriver to the Croton – Haverstraw Bay region (km 55 – km 63), causing a noticeable influx of larger, older larvae into the region on 14 March (Figs. 3A and 3B). Additional details of the association between larval tomcod and the 1.0‰ salt front are in Dew and Hecht (1994*a*).

Except for the temporary disruption on 4 April 1976, the 1975 and 1976 size gradients persisted until about mid-May (Tables 1 and 2), encompassing the larval, post-larval, and early juvenile life stages. In mid-May 1976, the gradient appeared to be breaking down (Fig. 3E). By 5 June 1976 the March–May size gradient had been reversed, and the mean length of upriver tomcod was now greater than that of downriver tomcod (Fig. 3F).

A plot of the ratio of upriver[2] fish length to downriver fish length in 1976 suggests that the process of gradient reversal began in late April – early May (Fig. 4). Prior to 1 May the ratio fluctuated without trend, largely in response to variations in freshwater flow. After 1 May, the ratio underwent a systematic trend toward reversal, which occurred after the 3rd week in May. It is of interest to note that the high-flow mixing of 4 April caused the ratio to approach 1.0 (complete mixing); but by 10 April, after freshwater flow had abated and ocean-derived salinity had intruded far upriver (Fig. 3C), the ratio had rebounded sharply (to 0.56) from its well-mixed state (Fig. 4). These dynamics suggest that the tomcod size gradient was maintained by water-mass movements that transported fish upriver according to their size and swimming ability. Larger tomcod, swimming more effectively against the upriver movement of the salt wedge, maintained their positions seaward of the smaller tomcod. This longitudinal size distribution persisted while the larger fish preferred to remain downriver.

[2] Upriver and downriver, as used here, refer to those fish upriver or downriver of the population epicenter, defined as the tomcod density-weighted average kilometre of the population on any given sampling date (Dew and Hecht 1994*a*). Inasmuch as the epicenter moved in association with the 1.0‰ salt front, the terms upriver and downriver here do not refer to fixed locations.

Table 1. Results of nonparametric Kruskal–Wallis tests and multiple comparisons of pooled mean lengths of Atlantic tomcod larvae among river regions by survey date in 1975.

River region	River kilometre	9 Mar.	16 Mar.	23 Mar.	29 Mar.	6 Apr.	12 Apr.	20 Apr.	26 Apr.	3 May	10 May
					Mean larval total length (mm)						
	97										
Pollepel Island	93	7.31*z*									
	90										
	87		7.23*z*								
	84										
Con Hook	80	7.29*z*									
	77										
	74										
	71										
Indian Point	68	7.65*y*		7.26*z*							
	64		7.61*y*				11.87*z*				
Haverstraw	61	7.82*x*									
	58							15.54*z*			24.81*z*
Croton	55		7.89*x*		7.20*z*	10.57*z*	12.87*y*			20.64*z*	
	52										
Tappan Zee Bridge	48						12.79*y*	14.61*zy*			
	45										
	42								17.94*z*		
Piermont	39	8.16*x*	8.18*x*	7.91*y*		10.80*z*	13.40*y*	14.05*y*		21.63*y*	27.76*y*
	35										
Hastings	32										
Yonkers	29			8.78*x*	9.15*y*		12.97*y*	14.35*y*	18.02*z*		26.19*zy*
	26									23.05*x*	
Harlem River	23										
	19					12.30*y*					
George Washington Bridge	16		9.57*w*		10.37*x*			15.52*z*		24.92*w*	
	13										
79th Street boat basin	10			9.06*x*			14.65*x*		19.60*x*		30.29*x*
	6										
Lower Manhattan	3				10.39*x*			16.95*x*		25.77*w*	
Statue of Liberty	0										
					Statistical summary						
Total sample size		474	568	284	242	440	390	791	375	617	510
Density-weighted mean length (mm)		7.73	8.11	8.55	10.03	11.13	13.06	15.24	18.24	23.07	28.82
Levene's heteroscedasticity[a]											
Untransformed data		11.95*	33.00*	15.40*	4.76*	16.23*	1.13	4.56*	3.67*	3.15*	2.71*
Log-transformed data		9.19*	19.50*	10.23*	1.49	8.49*	0.68	1.88	2.22	0.50	7.14*

Note: Parametric Student–Newman–Keuls multiple-range tests were used where log transformation reduced heteroscedasticity of variances to a nonsignificant ($P > 0.05$) level. Mean lengths on a sampling date that share the same letter were not significantly different from one another. On all dates, larvae near the mouth of the estuary were significantly larger than those near the head of the estuary. (From Dew and Hecht 1994*b*. Reprinted with permission.)

[a]Asterisks denote significant heteroscedasticities ($P < 0.05$).

Variance in mean length

Despite the apparent stability of the mean-length gradients, the river-long distribution of the variance around each of the means (Tables 1 and 2) was systematically changing. Throughout March–May 1975 and 1976, the date-specific relationship between the means in Tables 1 and 2 and their associated variation was changing from positive to negative (Fig. 5). In March and early April the greatest variation in length was associated with the largest (downriver) fish, as shown by the positive regression-slope values. Because the size of the variance was not independent of the size of the mean, significant variance heterogeneity resulted, as determined by Levene's test (Dixon et al. 1990) (Tables 1 and 2). During March–April while the variance–mean slope remained positive, variance heterogeneity was reduced via log transformation of the length data (as is typical for

Table 2. Results of nonparametric Kruskal–Wallis tests and multiple comparisons of pooled mean lengths of Atlantic tomcod larvae among river regions by survey date in 1976.

River region	River kilometre	7 Mar.	14 Mar.	21 Mar.	28 Mar.	4 Apr.	10 Apr.	17 Apr.	24 Apr.	1 May	9 May	15 May
						Mean larval total length (mm)						
	97											
Pollepel Island	93											
	90											
	87		7.11*z*									
	84	7.20*z*										
Con Hook	80											
	77											
	74											
	71											
Indian Point	68		7.71*y*				8.22*z*					
	64											
Haverstraw	61		8.43*x*									
	58	7.33*z*					12.77*y*					
Croton	55		8.45*x*					16.32*z*				
	52				8.79*z*				19.56*z*			
Tappan Zee Bridge	48			7.81*z*							29.29*z*	
	45									23.14*z*		
	42											
Piermont	39				8.87*z*		13.48*x*					36.16*z*
	35	7.35*z*										
Hastings	32			8.57*y*	9.21*y*				20.39*y*			
Yonkers	29		8.02*y*		9.65*x*					25.81*y*	31.56*y*	
	26	7.27*z*										
Harlem River	23	7.85*y*		8.96*x*		11.02*z*					33.81*yx*	37.84*z*
	19						14.60*w*	17.34*y*		26.96*y*		
George Washington Bridge	16			9.60*w*	10.01*x*	11.24*zy*						38.69*zy*
	13								22.38*x*			
79th Street boat basin	10	8.49*x*				12.14*y*					36.82*x*	
	6							19.39*x*		29.38*x*		39.84*y*
Lower Manhattan	3			10.52*w*		11.70*zy*						
Statue of Liberty	0					11.12*zy*						
						Statistical summary						
Total sample size		785	642	1177	802	591	1219	726	548	740	263	435
Density-weighted mean length (mm)		7.95	8.23	8.86	9.32	11.40	13.20	16.83	20.26	26.34	32.18	38.80
Levene's heteroscedasticity[a]												
Untransformed data		28.49*	14.02*	40.71*	7.24*	15.13*	2.86*	4.58*	2.76	0.85	3.34*	5.25*
Log-transformed data		17.98*	11.51*	26.80*	4.09*	10.52*	1.24	0.89	2.64	4.23*	6.30*	8.48*

Note: Parametric Student–Newman–Keuls multiple-range tests were used where log transformation reduced heteroscedasticity of variances to a nonsignificant ($P > 0.05$) level. Mean lengths on a sampling date that share the same letter were not significantly different from one another. On all dates except 4 April, larvae near the mouth of the estuary were significantly larger than those near the head of the estuary. (From Dew and Hecht 1994*b*. Reprinted with permission.)

[a]Asterisks denote significant heteroscedasticities ($P < 0.05$).

such data). Near the beginning of May, however, after the variance–mean slope had become negative (the greatest variance was now associated with the smallest means), log transformation perversely increased variance heterogeneity rather than reducing it (Tables 1 and 2). Movement of the largest age-0 tomcod from downriver into upriver regions occupied by the smaller fish apparently altered the variance structure so that log transformation, which affects large and small numbers differently, exacerbated the variance inequality between river locations.

Because of the orderly length gradient along the longitudinal axis of the river, the association between variance and fish length was also an association between variance and river kilometre. In March and April 1976, variation in length generally increased in a seaward direction (Figs. 3A–3C). This trend was reversed in May (Figs. 3D and 3E), perhaps because the largest tomcod were now actively migrating upriver.

A more complete picture of the distribution of variance during March–May 1975 and 1976 was obtained by using

Fig. 3. Changes in the longitudinal distribution of mean length, the variation in mean length, and the population density of age-0 tomcod in 1976, in relation to movements of the 1.0‰ salt front (vertical line). (A–C) There was a sharp decrease in mean length, variation in length, and population density upriver of the salt front in March and April. (D and E) In early to mid May, the variation in length began to increase moving upriver. (F) In early June, mean length itself began to increase moving upriver. Solid circles, mean length of larvae by sampling station (n = 785, 642, 1219, 263, 435, and 346 for A–F, respectively); solid lines, mean length of smallest and largest 20% of larvae (A–C) or postlarvae (D–F); broken lines, larval population density.

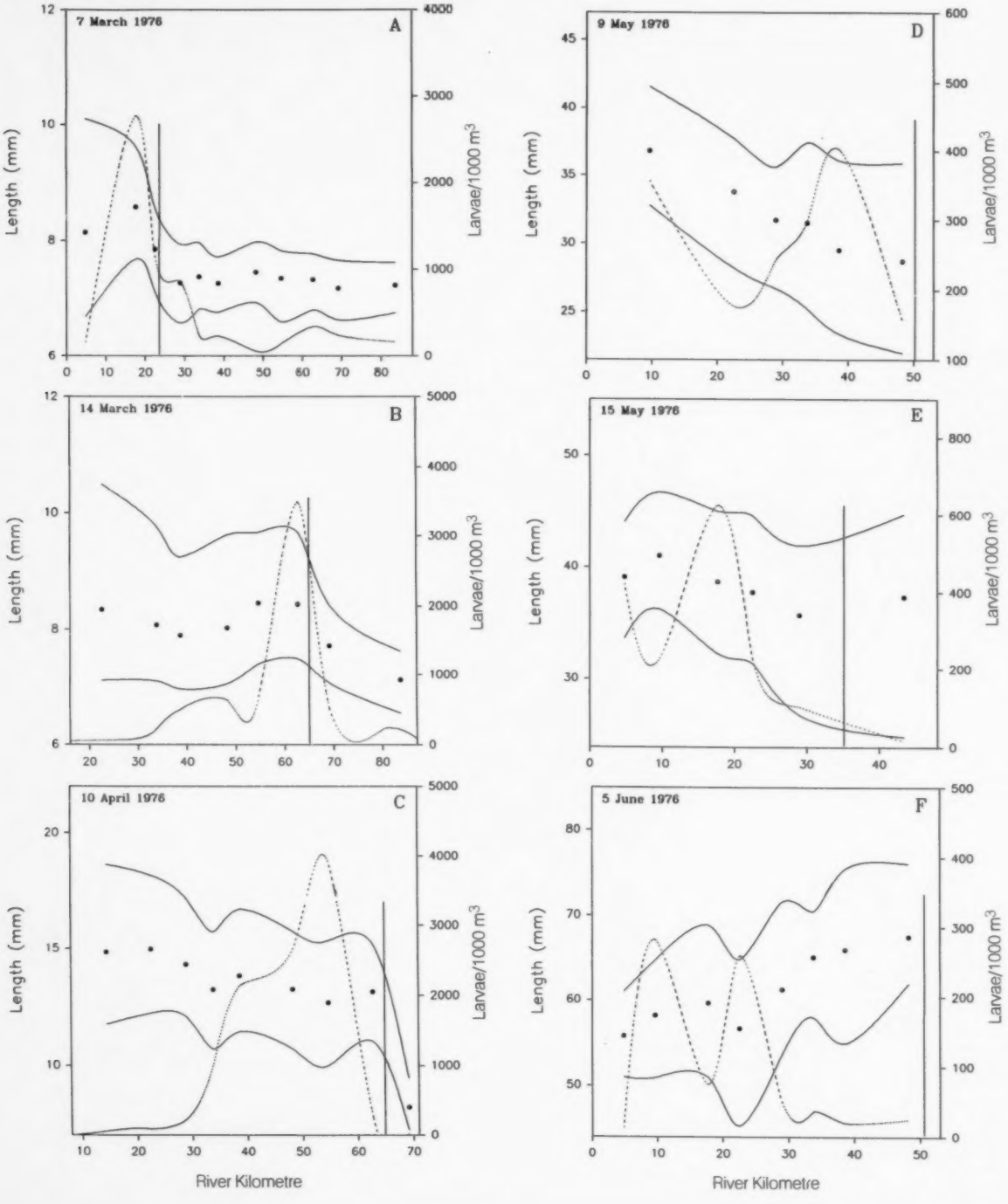


Fig. 4. A ratio comparing the mean length of fish collected from the upriver and downriver tails of the age-0 tomcod population distribution on each sampling date from 7 March to 13 June 1976. Presumably, the cumulative effect of larger fish moving upriver caused the ratio to exceed 1.0 after 21 May.

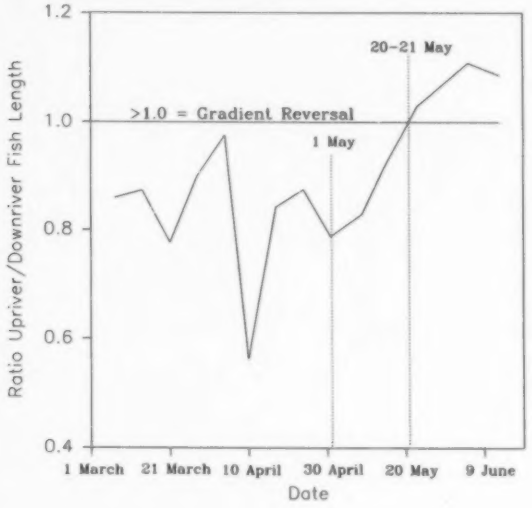
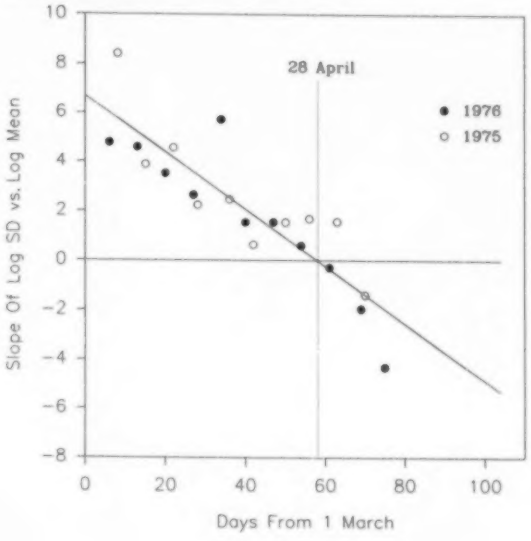


Fig. 5. The trend in the relationship between location-specific mean lengths (Tables 1 and 2) and their standard deviations on sampling dates in 1975 and 1976. The magnitude of the standard deviations was positively associated with that of the means in March and April. After late April the two were negatively associated, indicating that the highest variation in length was now associated with the smaller fish.



response–surface analysis to model variance as a function of time and river location in kilometres. Changes in the variance distribution served as a proxy for changes in fish distribution, i.e., larger tomcod were associated with higher variance. In March of both years (Fig. 6), the contours showed that variance increased in a seaward direction and was highest (lightest shading) at the time and location of the largest fish. In April of both years the contours became more vertical with time, suggesting that high-variance factors in the form of relatively large, age-0 tomcod were moving upriver, tending to equalize the variance among river locations. Consistent with the concept of upriver movement, variance heterogeneity declined to a non-significant level between mid-April and early May (Tables 1 and 2), as can be inferred from the relatively wide spacing of the variance isopleths in early May (Fig. 6).

Reversal of the variance gradient became obvious after the 1st week in May (day 68) of 1975 and 1976, several weeks before the reversal in mean length became apparent on 5 June 1976 (Fig. 3F). These observations led to the hypothesis that, in both years, the cumulative effect of the largest tomcod actively moving upriver during April caused an early May reversal in variance (square millimetres); for the less sensitive[3] parameter of mean length (millimetres), reversal did not become evident until late May. Consistent with the hypothesis of an April upriver migration by larger tomcod is the first appearance, in April, of tomcod on the intake screens of the Indian Point Nuclear Generating Station (km 69). As indicated by 5 years of impingement data (Fig. 7), the migration past km 69 typically begins in April and swells in magnitude throughout the spring as the tomcod, which spawn in December–January as age-0 fish, move toward the spawning grounds upriver of km 80.

[3] For a given data set, the addition of a few, relatively large fish increases the variance much more than the mean.

Growth

The March–May 1976 growth rate (G) of tomcod collected downriver (km 0 – km 31) was compared with that of tomcod collected upriver (km 32 – km 93) to see if the hypothesized upriver migration affected growth rates. For 6–7 weeks after the estimated hatching peak on 9 March (Dew and Hecht 1994*b*), the growth of upriver fish (G = 0.0254) was marginally slower (P = 0.048) than that of downriver fish (G = 0.0278). In late April, at a mean length of 20 or 21 mm, the growth rate of upriver fish increased (P = 0.001) to G = 0.0355, a rate significantly ($P < 0.001$) greater than that of downriver fish, which remained the same (Table 3, Fig. 8). The increase in the growth rate of upriver fish would be expected if substantial numbers of larger tomcod from downriver began to move upriver in late April.

A similar analysis was not possible for 1975, when sampling ended on 10 May (vs. 13 June in 1976) and the 22-mm cohort was the last length cohort with a complete density distribution through time (vs. 63 mm in 1976). From 7 through 22 mm in 1975, the growth of upriver (G = 0.0220) and downriver fish (G = 0.0216) was the same (P = 0.757) (Table 3).

Fig. 6. Isopleth contours showing the distribution of variance in space and time during March–May 1975 and 1976 (lighter shading denotes higher variance). In March there was a gradient of increasing variance moving downriver away from the location and time of hatching, i.e., the highest variance was associated with the larger, downriver fish. In April, variance continued to increase with time (x axis) as the fish grew larger, but the river-long gradient (y axis) had largely deteriorated. After the 1st week in May, the initial gradient had been reversed, and the highest variance was now associated with the smaller, upriver fish. The R^2 value is an overall measure of the model fit.

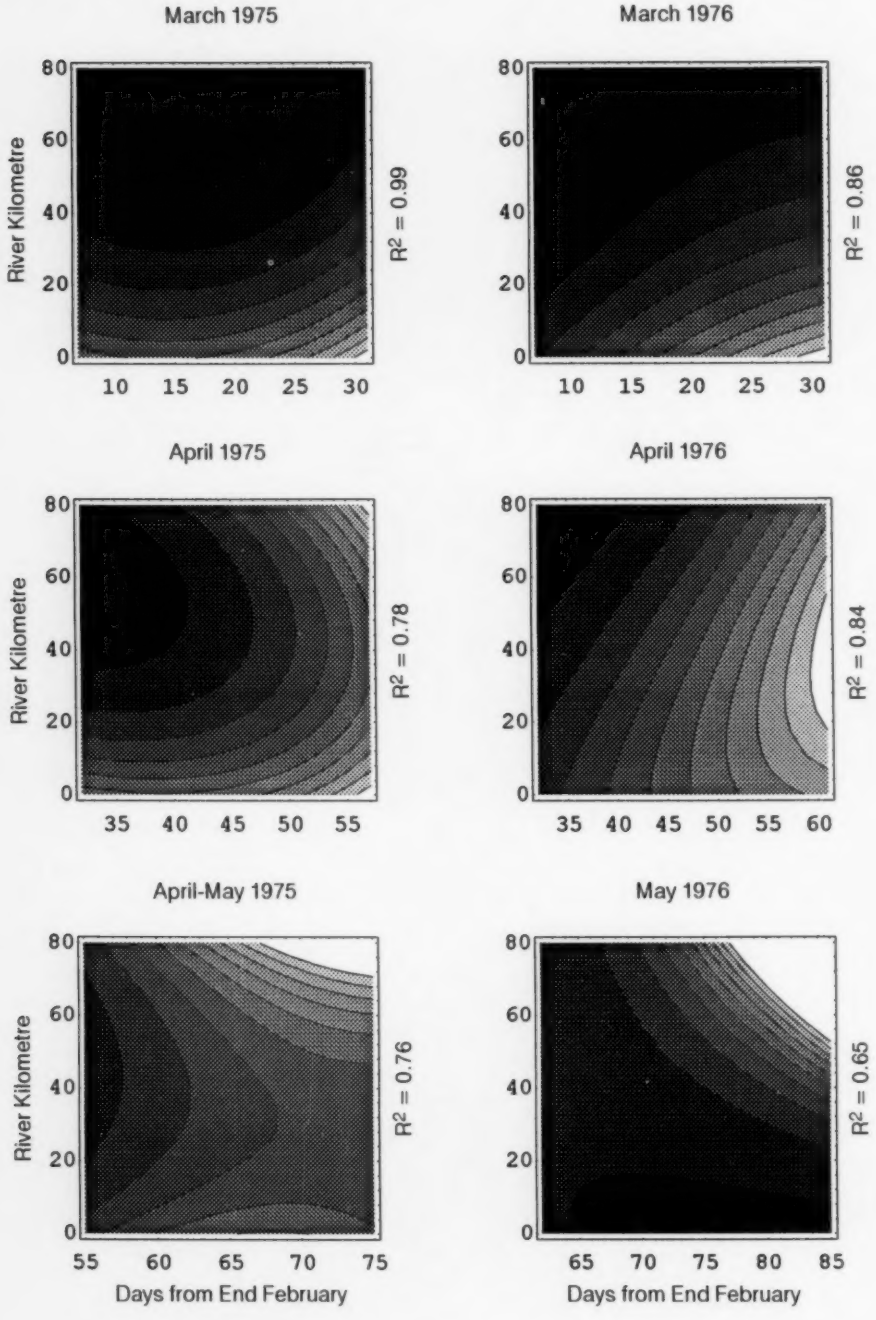
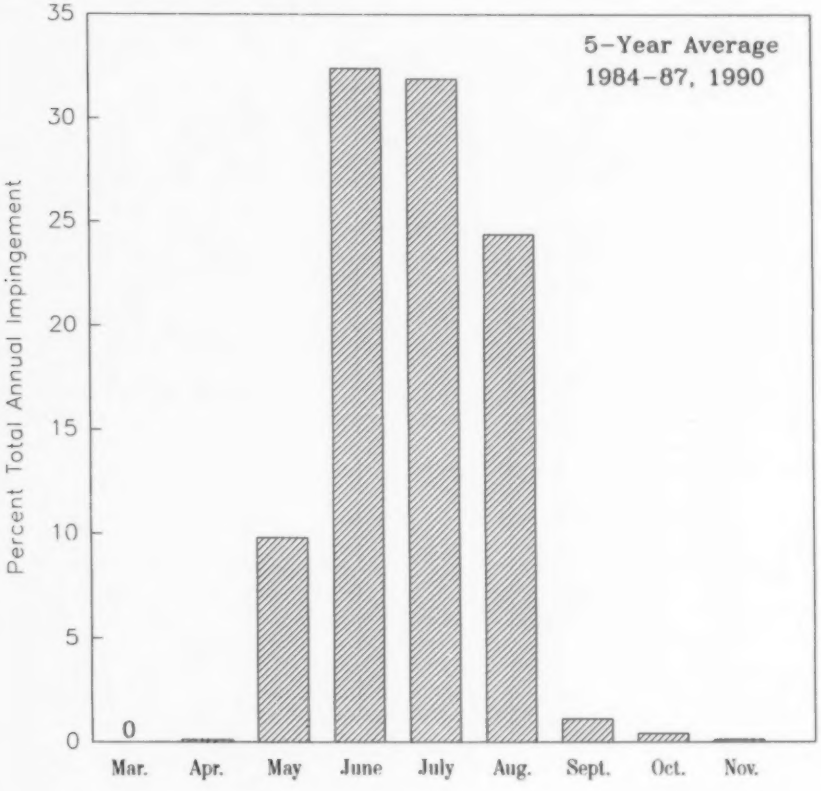


Fig. 7. Multiyear, seasonal trend in the impingement of young-of-the-year Atlantic tomcod at the Indian Point Nuclear Generating Station (km 69). Consistent with an upriver migration of tomcod >20 mm long, impingement began in April and increased to a maximum in June. This composite trend was drawn from 5 years for which data were readily available (1984–1987 and 1990).



Discussion

Given that the distribution of larval fish is controlled by both active behavior and passive transport (Norcross and Shaw 1984), it was not entirely unexpected that semiplanktonic, age-0 Atlantic tomcod in the lower Hudson River would be arranged in some sort of a nonrandom size gradient. Still, it was instructive to see the March–May persistence of this gradient throughout a 93-km stretch of the estuary under highly variable flow regimes in two different years and to observe the rapidity with which the gradient was reestablished after a high-flow episode of near-complete mixing in April 1976.

Elements of the two-layer estuarine transport system and aspects of tomcod behavior apparently acted in concert to distribute the fish along the bottom in a nonrandom, orderly, longitudinal progression of lengths throughout the intrusion region. The salt-intruded reach of a partially stratified estuary such as the Hudson River is characterized by net current flow near the bottom that is always in the upriver, landward direction, even though the currents at all depths reverse for some period of time within each tidal phase (Abood 1974). Under conditions of high freshwater flow, lower layer flow is in a landward direction during most of the tidal cycle, replacing the saline water entrained into the seaward-moving upper layer.

Tomcod hatch each year in the freshwater reach of the estuary (Dew and Hecht 1994*a*), where there is no lower layer flow and the net current movement is seaward at all depths (Abood 1974). Upon meeting the salt wedge that intrudes from the ocean, the less dense, seaward-moving freshwater moves upward along the face of the wedge and becomes part of the upper layer flow characteristic of the intrusion region. Clearly, larval tomcod would be swept from the estuary if they did not move from the upper layer to the lower layer of the estuarine transport system. Data from Dew and Hecht (1994*a*, Fig. 8) suggest that this movement is effected by downwelling in the vicinity of relative km 0, which they defined as the location of the 1.0‰ salt front at any given time.

It is entirely likely that estuarine hydrodynamic forces dwarfed the ability of larval and early juvenile tomcod to maintain an absolute position along the longitudinal axis of the estuary. However, if young tomcod are

Table 3. Comparison of parameter estimates for the growth of age-0 Atlantic tomcod collected in 1975 and 1976 from the upriver, Hastings – Pollepel Island region (km 32 – km 93) versus tomcod from the downriver, Statue of Liberty – Yonkers region (km 0 – km 31).

	1976			1975	
	Upriver (7–20 mm)	Upriver (21–42 mm)	Downriver (7–50 mm)	Upriver (7–22 mm)	Downriver (7–22 mm)
Intercept	1.8034	1.4189	1.8248	1.8734	1.9207
Slope (G)	0.0254	0.0355	0.0278	0.0220	0.0216
S_b[a]	0.0014	0.0022	0.0004	0.0010	0.0010
S_y[b]	0.0628	0.0549	0.0458	0.0622	0.0653
R^2	0.967	0.934	0.993	0.971	0.968
df	12	19	42	14	14

[a]Standard error of the regression coefficient.
[b]Standard error of the estimate.

positively rheotropic as are the adults (Fortin et al. 1990), and if swimming ability increases with fish size, then the larger fish would naturally maintain a relative position within the salt-intruded reach that was seaward and upcurrent of the smaller fish. The result would be a longitudinal size hierarchy that would produce a gradient such as the one we observed during March–May 1975 and 1976. A similar size gradient was reported for age-0 tomcod during July in the St. Lawrence estuary, but the inverse (larger tomcod upriver of smaller ones) was reported for June (Laprise and Dodson 1990). It is possible that reliance on a fixed location to sample a large-scale, longitudinal distribution contributed to the ambiguity of the St. Lawrence findings. In the Hudson River, the size gradient weakened and eventually was reversed, presumably because the larger tomcod downriver grew to lengths greater than 20 mm and then began to migrate upriver.

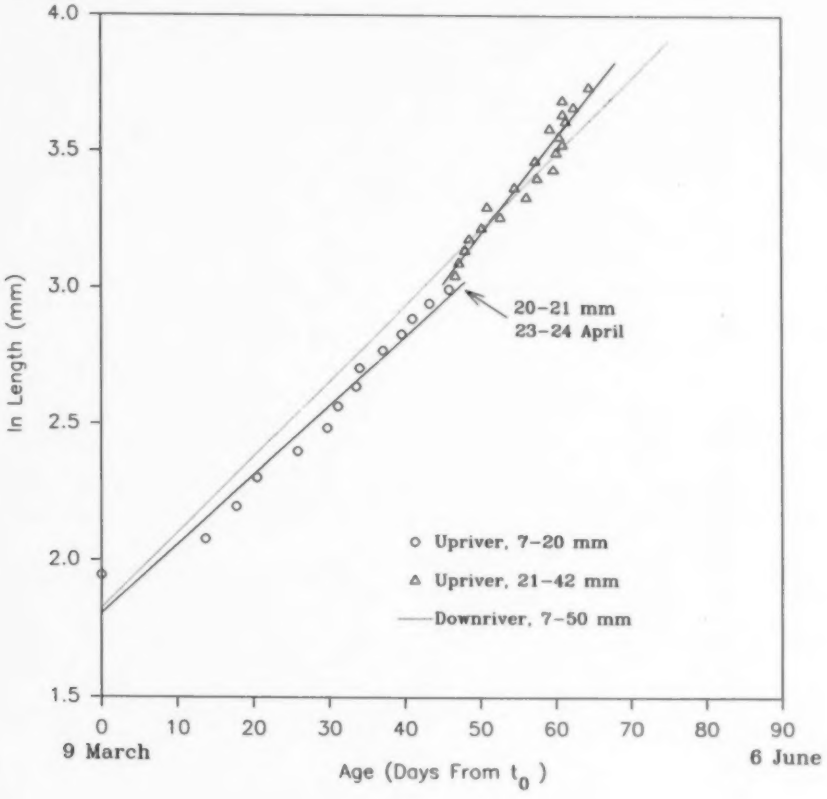
The size-specific distribution and movements discussed here for age-0 tomcod have some bearing on the research of other Hudson River investigators. For example, March–May sampling by investigators from the electric utilities, which excluded the river region seaward of the Harlem River (km 22), failed to encompass approximately 29% of the total standing crop of age-0 tomcod in 1975 and 45% in 1976 (Dew and Hecht 1994*a*). The distinctly nonrandom distribution imposed on the tomcod population by the estuarine transport system ensured that the excluded tomcod seaward of km 22 were the largest and probably the oldest fish in the age-0 population. Clearly, the relevance of mortality and growth estimates (e.g., McLaren et al. 1988) is diminished if the largest and oldest members of the target population are systematically excluded from analysis.

In other work, a late April 1976 increase in the mortality of tomcod greater than 24 or 25 mm was attributed to the spring renewal of feeding by yearling striped bass that had overwintered in the estuary (Dew 1991; Dew and Hecht 1994*b*). In light of the present analysis, it is equally likely that the perceived increase in mortality was an artifact caused by a size-specific migration of larger fish from the sampling region. Abundance trends from the weekly, daytime sampling of Dew (1991) and Dew and Hecht (1994*b*) did not show such a migration, perhaps because it was a low-level, continuous phenomenon or because it occurred primarily at night. Indeed, abundance trends are not likely to reveal the early stages of an upriver movement in which the only participants were fish greater than 20 mm in length.

It is evident that Atlantic tomcod have evolved to take advantage of the retentive nature of estuarine transport systems. However, for age-0 tomcod in the Hudson River, the gain in survival that might have accrued from being retained within the estuary has been at least partially dissipated by entrainment and impingement mortality at the cooling-water intakes of power plants. In particular, the Bowline (km 60), Lovett (km 68), and Indian Point (km 69) generating stations collectively withdraw 123.4 $m^3 \cdot s^{-1}$ of Hudson River water when operating at full capacity (Hutchison 1988). Entrainment and impingement of age-0 tomcod occur each year at these three cooling-water intakes during (*i*) the downriver transport of larvae from freshwater hatching areas to the salinity-intrusion zone; (*ii*) the upriver and downriver transport of larvae and early juveniles in association with movements of the 1.0‰ salt front; and (*iii*) the active, upriver migration of juveniles greater than 20 mm in length.

The frequency and duration of exposure of tomcod larvae to entrainment at power-plant intakes is dependent, in large part, on the magnitude and variability of March–May freshwater flow. Near the end of hatching in mid-March 1976, when the estimated freshwater flow had temporarily fallen to 447 $m^3 \cdot s^{-1}$, saltwater intrusion transported peak larval densities landward into the high-vulnerability zone upriver of km 54 (Boreman and Goodyear 1988). Equations from Dew and Hecht (1994*a*) predict that, after hatching, the bulk of the age-0 tomcod population will be transported upriver into the high-vulnerability zone each time the freshwater flow declines to approximately 400 $m^3 \cdot s^{-1}$ or less, or when the 1.0‰ salt front intrudes to km 72 or beyond. Therefore, entrainment impact, largely dependent on the passive transport of larvae and post-larvae, is

Fig. 8. Comparison of upriver (km 32 to km 93) and downriver (km 0 to km 31) growth of age-0 tomcod in 1976. The rate of growth for downriver fish did not change during March–May. At a length of 20 or 21 mm in late April, the growth of upriver fish increased significantly ($P = 0.001$), apparently because larger fish were moving into the region from downriver.



expected to be highest in those years when March–May flows are lowest.

Impingement is more dependent on the presence of actively migrating juveniles sufficiently large (≥25 mm) to be caught with some regularity on the 9.5-mm square-mesh intake screens. On the basis of the present data, I have hypothesized an upriver migration of juvenile tomcod greater than 20 mm in length beginning in April. If true, I would expect impingement of age-0 tomcod at km 60 – km 69 to begin each year in late April or early May and to increase throughout May and June as the migration progressed and as spring flows subsided to levels below 400 $m^3 \cdot s^{-1}$. The multiyear pattern evident for March–May impingement of age-0 tomcod at Indian Point (km 69) is generally consistent with this expectation (Fig. 7).

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Accumulation of trace metals (Cd, Pb, Cu, Zn) in Arctic char (*Salvelinus alpinus*) from oligotrophic Alpine lakes: relation to alkalinity

G. Köck, R. Hofer, and S. Wögrath

Abstract: We studied Arctic char (*Salvelinus alpinus*) from five oligotrophic Alpine lakes in northern Tyrol, Austria, that differed in altitude (929–2796 m above sea level), pH (pH 5.4–8.3), and alkalinity (1.3–2750 μequiv.·L^{-1}). Concentrations of Cd, Pb, Zn, and Cu were measured in the water and in the kidney of fish, allowing the calculation of metal bioconcentration factors between water and fish kidney. Despite relatively low Cd and Pb concentrations in the water (Cd, 0.1 μg·L^{-1}; Pb, 0.5 μg·L^{-1}, dissolved), metal concentrations in the kidneys of fish from the most acidic lake (Cd, 123 μg·g^{-1}; Pb, 19 μg·g^{-1}, dry weight) were comparable with those of fish from waters polluted by direct discharge of metals. Concentrations of Cd and Pb in the kidney were negatively correlated with the alkalinity of the lakes. Comparison with data from other studies confirms the predictive utility of water alkalinity for estimating Cd accumulation in fish from oligotrophic lakes. The correlation between bioconcentration factors and alkalinity was negative for Cd and Pb, positive for Zn, and nonsignificant for Cu.

Résumé : Nous avons étudié des ombles chevaliers (*Salvelinus alpinus*) provenant de cinq lacs alpins oligotrophes du nord du Tyrol (Autriche), qui différaient par l'altitude (929–2796 m au-dessus du niveau de la mer), le pH (5,4–8,3) et l'alcalinité (1,3–2750 μequiv.·L^{-1}). Les concentrations de Cd, Pb, Zn et Cu ont été mesurées dans l'eau et dans les reins des poissons, ce qui a permis de calculer les facteurs de bioconcentration des métaux de l'eau aux reins des poissons. Malgré des concentrations relativement faibles de Cd et de Pb dans l'eau (Cd, 0,1 μg·L^{-1}; Pb, 0,5 μg·L^{-1}, état dissous), les concentrations de métaux dans les reins des poissons provenant des lacs les plus acides (Cd, 123 μg·g^{-1}; Pb; 19 μg·g^{-1}, poids sec) étaient comparables à celles des poissons provenant d'eaux polluées par des rejets directs de métaux. Les concentrations de Cd et de Pb dans les reins étaient négativement corrélées à l'alcalinité des lacs. La comparaison des résultats avec les données d'autres études confirme la valeur prédictive de l'alcalinité de l'eau pour estimer l'accumulation de Cd chez les poissons des lacs oligotrophes. La corrélation entre les facteurs de bioconcentration et l'alcalinité était négative pour le Cd et le Pb, positive pour le Zn et non significative pour le Cu.

[Traduit par la Rédaction]

Introduction

Most of the high mountain lakes of the Alps are situated within crystalline watersheds above 2000 m above sea level (asl). Despite extreme environmental conditions (ice covered for 7–9 months, oligotrophic, low pH, and low alkalinity) some remote Austrian high mountain lakes are populated by fish that were introduced by man during the 16th century. These lakes are not stocked or fished. Such old fish populations represent a valuable genetic pool worth preserving. In a preliminary study we found high concentrations of Cd and Pb in fish from an Austrian high mountain lake far from pollution sources.

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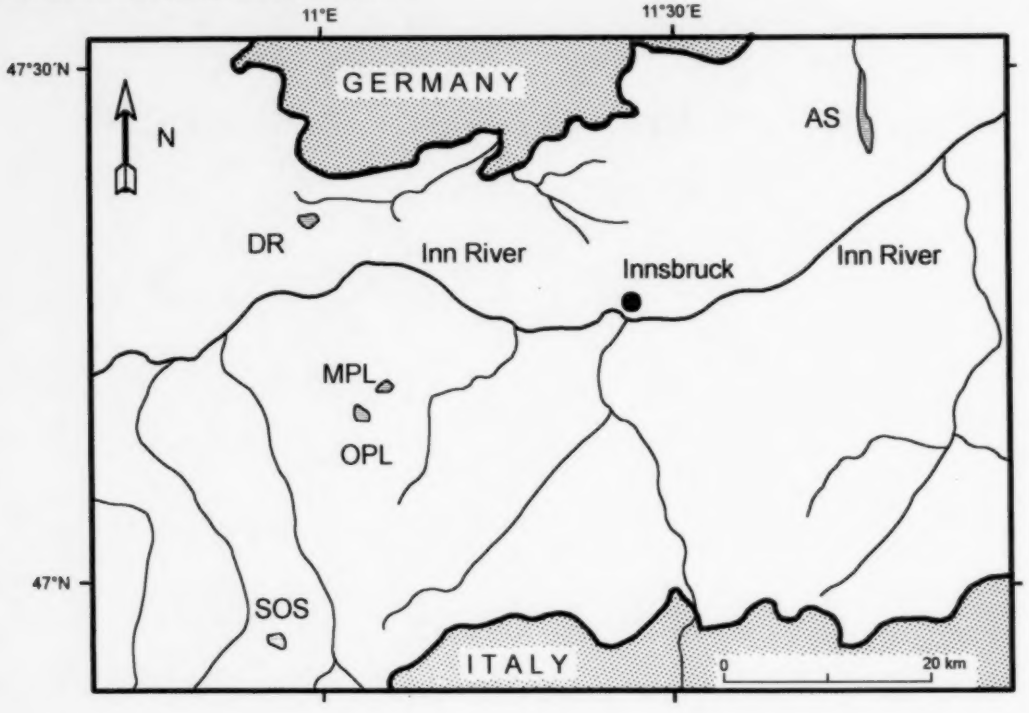
The uptake of trace metals by fish is highly dependent on the physical and chemical characteristics of the water. The toxicity of divalent metals is greatest in soft water with low concentrations of dissolved organic carbon (DOC) and suspended particles (Alabaster and Lloyd 1980; Welsh et al. 1993), characteristics of many lakes of the Alps (Psenner 1994). The low buffering capacity of the water, atmospheric deposition of sulfuric and nitric aerosols, and accumulation of CO_2 under ice cover can cause acidification of the water and subsequent changes in the speciation of certain metals (Campbell and Stokes 1985; Psenner 1989; Steinnes 1990). Geogenic leaching and atmospheric deposition contribute to the concentrations of metals in the water (Haines 1981; Nelson and Campbell 1991).

The impact of pH and water hardness on the toxicity of trace metals to fish has been well studied under laboratory conditions. Fewer data are available, however, for field conditions, making it difficult to provide quantitative correlations between the accumulation of trace metals in fish

Fig. 1. Location of lakes studied. AS, Achensee; DR, Drachensee; MPL, Mittlerer Plenderlesee; OPL, Oberer Plenderlesee; SOS, Schwarzsee ob Sölden.



organs and water parameters such as pH, hardness, or alkalinity. Furthermore, most of the research so far has been carried out in waters of considerably higher ionic strength than has been reported for many oligotrophic high alpine lakes.

We examined the relation of metal concentration in fish to selected physicochemical features of high mountain lakes. In consequence, we investigated the accumulation of metals in Arctic char, *Salvelinus alpinus*, from several oligotrophic lakes spanning a wide range in alkalinity and pH. This paper is the first of a series on the impact of an extreme environment on the pathology and physiology of fish in high mountain lakes.

Materials and methods

Between summer 1992 and autumn 1993, Arctic char were sampled from five Alpine lakes in northern Tyrol, Austria (Fig. 1), spanning broad ranges in altitude (929–2796 m asl) and physicochemical water characteristics (Tables 1 and 2). Both the amount of precipitation and the main wind directions do not differ among the locations of the lakes (Fliri 1975); thus, a significant spatial gradient in atmospheric metal load appears to be unlikely in the study area.

Fish were caught with gill nets of 12.5-, 16-, 22-, 25-, and 30-mm mesh size in summer and with hook and line during the ice-covered period. Fish were quickly transported to the laboratory, killed by a blow on the head, and dissected. Otoliths and opercula were removed, cleaned, and used for age determination. Because of age-dependent metal accumulation (Köck et al. 1991) only fish from 6 to 10 years of age were analyzed (Table 3). The kidney is known as one of the major sites of metal accumulation in fish (Dallinger et al. 1987) and was therefore used as a metal monitoring organ. Kidney samples were transferred into washed, preweighed 10-mL polypropylene tubes. After drying (at 60°C to constant weight), the samples were digested by addition of HNO_3 and H_2O_2 in a microwave oven. After complete digestion (Merck Suprapure nitric acid) the digestates were diluted with double-deionized water to a final volume of 10 mL. Metal analysis was performed by graphite furnace atomic absorption spectrophotometry (GFAAS) with Zeeman background correction. To reduce chemical interference, $Pd(NO_3)_2$ matrix modifier was used. Quantification was achieved by reference to calibration plots obtained in standard solutions in a similar HNO_3 matrix.

Water samples from different depths (from surface to bottom in 2-m intervals; volume-weighted averaging for all chemical data) were collected by a polyacrylic Schindler sampler at least once in each season and biweekly during the snowmelt and ice breakup period (end of April to end of July). Metal concentrations in acidified double-deionized water that was held in the sampler for 15 min were below detection limits, indicating that metal contamination by the water sampler was not a problem. The samples (except those analyzed for pH, conductivity, and acid-neutralizing

Table 1. Physical characteristics of lakes studied.

Lake	Location	Bedrock	Altitude (m)	Surface area (ha)	Watershed area (ha)	Maximum depth (m)	Volume (1000 m^3)	Ice-covered period (months)
Schwarzsee ob Sölden (SOS)	46°57′57″N 10°56′46″E	Old crystalline basement complex (granitic gneisses)	2796	3.5	18	18	292	9.5
Mittlerer Plenderlesee (MPL)	47°12′30″N 11°03′19″E	Old crystalline basement complex (granitic gneisses)	2317	1.6	8	5.7	49.6	7–8
Oberer Plenderlesee (OPL)	47°12′13″N 11°02′13″E	Old crystalline basement complex (granitic gneisses)	2344	2.1	97	7.5	69	7–8
Drachensee (DR)	47°21′30″N 10°56′54″E	Northern calcareous Alps (limestones)	1874	5.0	188	24	437.4	7–8
Achensee (AS)	47°27′30″N 11°42′30″E	Northern calcareous Alps (limestones)	929	680	10.5×10^3	133	481×10^3	0–1

Table 2. Chemical characteristics of lakes studied.

Lake	n	Cond. ($\mu S\cdot cm^{-1}$)	pH	ANC ($\mu equiv.\cdot L^{-1}$)	$[Ca^{2+}]$ ($mg\cdot L^{-1}$)	$[Na^{+}]$ ($mg\cdot L^{-1}$)	$[K^{+}]$ ($mg\cdot L^{-1}$)	$[Cl^{-}]$ ($mg\cdot L^{-1}$)	[Cd] ($\mu g\cdot L^{-1}$)	[Pb] ($\mu g\cdot L^{-1}$)	[Cu] ($\mu g\cdot L^{-1}$)	[Zn] ($\mu g\cdot L^{-1}$)	[Mn] ($\mu g\cdot L^{-1}$)	[Fe] ($\mu g\cdot L^{-1}$)	[Al] ($\mu g\cdot L^{-1}$)	DOC ($mg\cdot L^{-1}$)
SOS	18	12.7	5.4	1.3	1.02	0.25	0.10	0.13	0.114	0.504	1.19	2.68	6.76	6.74	40.0	0.62
		(1.8)	(4.8–6.2)	(−7.8–+7.7)	(0.16)	(0.03)	(0.02)	(0.09)	(0.097)	(0.406)	(0.08)	(0.22)	(1.38)	(4.28)	(10.0)	(0.28)
MPL	8	11.2	5.9	18.3	1.1	0.26	0.24	0.10	0.068	0.325	0.75	2.22	6.66	6.22	<10	0.77
		(1.1)	(5.3–6.6)	(1.5)	(0.1)	(0.01)	(0.03)	(0.02)	(0.019)	(0.202)	(0.55)	(0.25)	(3.1)	(2.88)		(0.25)
OPL	12	30.2	7.1	100.3	3.11	0.41	0.44	0.11	0.036	0.212	0.62	1.85	2.18	0.65	<10	0.66
		(4.6)	(6.1–7.3)	(18.6)	(0.13)	(0.06)	(0.04)	(0.03)	(0.016)	(0.20)	(0.20)	(0.53)	(1.5)	(0.4)		(0.08)
DR	6	138.1	8.3	1357	25.2	0.08	0.09	0.20	0.036	0.813	0.87	1.12	0.26	0.42	<10	0.86
		(9.3)	(7.8–8.6)	(176)	(0.8)	(0.02)	(0.02)	(0.06)	(0.016)	(0.605)	(0.53)	(0.31)	(0.01)	(0.14)		(0.13)
AS	6	274.6	8.2	2749	34.8	0.61	0.30	0.98	0.022	0.316	1.89	1.02	0.52	0.38	<10	1.15
		(2.7)	(8.0–8.6)	(23)	(0.5)	(0.02)	(0.04)	(0.17)	(0.011)	(0.08)	(1.72)	(0.82)	(0.18)	(0.29)		(0.32)

Note: Values are given as the means of measurements carried out during the whole experimental period with standard deviations or ranges given in parentheses.

Table 3. Sampling frequency (summer 1992 – autumn 1993) and biometric data of fish from the lakes studied.

Lake	Sampling frequency	Body length (cm)	Body weight (g)	Condition factor[a]	Age (years)	Sex ratio (male/female)
SOS	6	23.2 (18.0–28.0)	101.0 (49.1–148.4)	0.80 (0.63–0.99)	8.2 (6–10)	31/35
MPL	1	24.8 (21.5–27.8)	125.3 (75.4–165.7)	0.81 (0.74–0.91)	8.4 (6–10)	2/5
OPL	9	16.5 (14.0–21.0)	31.5 (17.3–16.6)	0.68 (0.52–0.77)	7.2 (6–10)	11/17
DR	1	21.4 (19.0–25.3)	67.4 (49.5–105.9)	0.67 (0.59–0.74)	7.0 (6–10)	1/13
AS	2	32.4 (17.3–65.0)	367.0 (38.8–2300)	0.78 (0.59–0.92)	7.2 (6–19)	14/6

Note: Values are means, with ranges given in parentheses.

[a]Condition factor (CF) was calculated according to the formula $CF = 100\ W/L^3$, where W is body weight in grams and L is total body length in centimetres.

Table 4. Results from analysis of National Research Council Canada standard reference materials (TORT-1, lobster hepatopancreas; SLRS-2, riverine water).

Standard reference material	Cd	Pb	Cu	Zn	Mn	Fe
TORT-1						
Certified value ($\mu g \cdot g^{-1}$)	26.3±2.1	10.4±2.0	439±22	177±10	—	—
Measured value ($\mu g \cdot g^{-1}$)	26.0±2.9	10.5±1.8	430±24	176±8	—	—
Recovery (%)	99	101	98	99	—	—
SLRS-2						
Certified value ($\mu g \cdot L^{-1}$)	0.028±0.004	0.129±0.011	2.76±0.17	3.33±0.15	10.1±0.3	129±7
Measured value ($\mu g \cdot L^{-1}$)	0.028±0.006	0.128±0.014	2.85±0.19	3.48±0.14	9.9±0.4	124±9
Recovery (%)	100	100	103	105	98	96

Note: Values are given as means ± SD ($n = 3$).

capacity) were filtered in the field using a precleaned polypropylene syringe fitted with a 0.45-μm Millipore cellulose acetate filter, and collected in acid-washed polyethylene bottles. Samples for metal analysis were acidified for preservation.

Conductivity was determined with a WTW LF 191 conductimeter and pH with a Schott CG 818 pH meter equipped with an Ingold pH electrode recommended for pH measurements in water of low ionic strength. Acid-neutralizing capacity (ANC) was determined by Gran titration. Calcium, sodium, and potassium were analyzed with a DIONEX DX-100 ion chromatograph. Aluminum determination was carried out by a photometric method with Alizarin S. DOC and chloride were determined with a CENCO autoanalyzer. Cadmium, lead, copper, zinc, manganese, and iron were determined in acidified subsamples by GFAAS. Because of their low concentration in the water samples, cadmium and lead were measured by using the sample concentration option of the employed Hitachi Z-8200-GFAAS instrument. This option provides the possibility of concentrating a sample by repeating the drying stage at each sample injection up to 25 times.

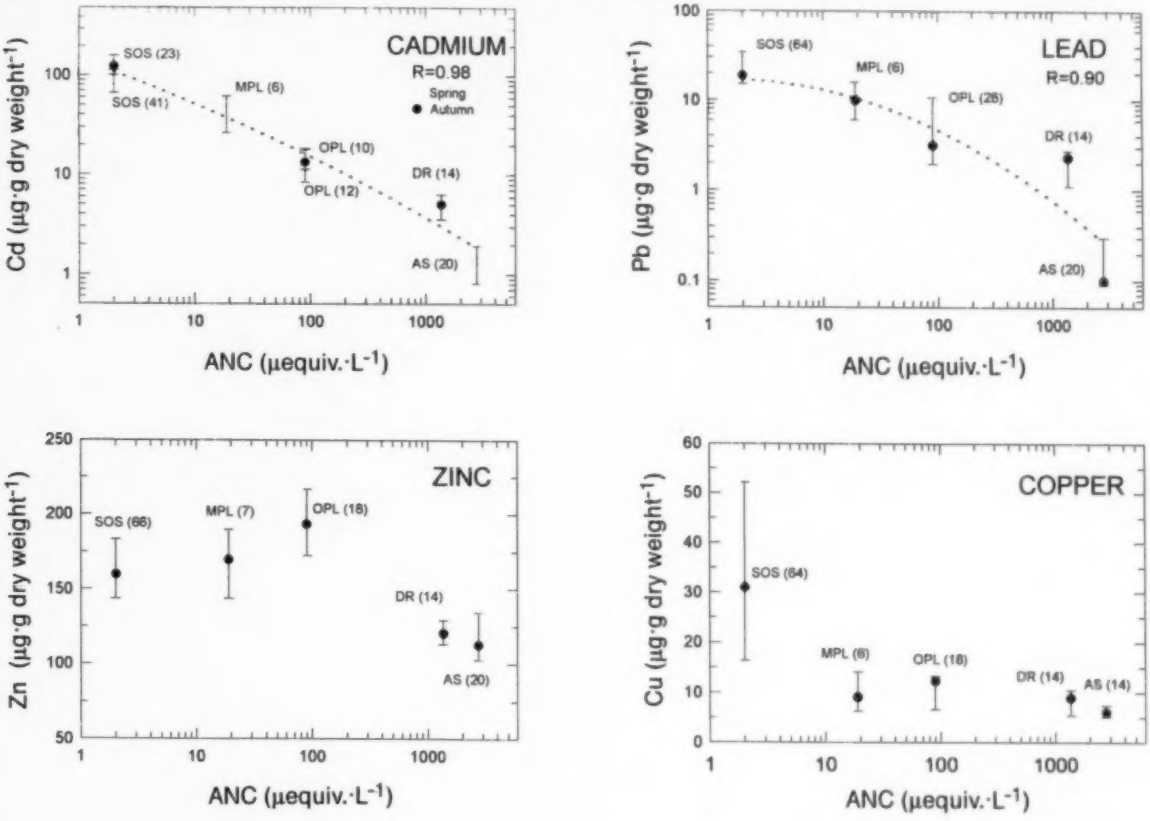
Quality assurance protocols for determination of metal concentrations in tissues and water samples included analysis of National Research Council Canada standard reference materials (TORT-1 lobster hepatopancreas, SLRS-2 riverine water), procedural blanks, and triplicate determinations (Table 4). In all cases, metal concentrations in procedural blanks were below detection limits. For control purposes, the Cd and Pb concentrations of a few water samples were checked by staff of another laboratory; their results did not differ significantly from ours. Bioconcentration factors (BCF) were used as a measure of the metal availability in the lakes to fish (Wiener and Giesy 1979). BCFs between water and kidney of Arctic char were calculated according to the formula

$$\mathrm{BCF} = \frac{C_{\mathrm{org}}}{C_{\mathrm{water}}}$$

where C_{org} is the metal concentration in the organism (dry weight) and C_{water} is the metal concentration in water. Metal concentrations in fish are expressed on a dry weight basis and can be transformed to wet weight concentrations by multiplying by 0.19 ± 0.2.

Data were tested by regression analysis and Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks. Multiple comparisons were made with Dunn's test. Statistical significance was assigned at $p \leq 0.05$. Because of

Fig. 2. Accumulation of Cd, Pb, Zn, and Cu in the kidney of *Salvelinus alpinus* in relation to lake alkalinity (ANC). Median and percentiles (25 and 75%) and number of fish (in parentheses) are given. Regression equations: log Cd = 2.16 − 0.41 log ANC − 0.04 log ANC^2; log Pb = 1.22 + 0.06 log ANC − 0.17 log ANC^2.



non-normal distribution of data, median values and percentiles (25 and 75%) are given.

Results

Water chemistry

There were major differences between the lakes for pH (5.4–8.3), ANC (1.3–2750 µequiv.·L^{-1}), conductivity (11–275 µS·cm^{-1}), and Ca concentration (1–35 mg·L^{-1}) of the water (Table 2). Considerable seasonal fluctuations of pH were observed in high-altitude lakes resulting from accumulation of CO_2 during winter, particularly in lakes with low buffering capacity. The Na concentration was extremely low in Drachensee (DR), whereas the hard water of Achensee (AS) contained the highest concentrations of sodium and chloride. Calcium concentrations and pH of the lakes were positively correlated to alkalinity. In all of the lakes studied the concentrations of trace metals were relatively low. A significant negative correlation to alkalinity was found only for Cd (R = 0.99) and Zn (R = 0.99). Concentrations of Mn and Fe were high in the two low-pH lakes (Schwarzsee ob Sölden (SOS) and Mittlerer Plenderlesee (MPL)), whereas the Al concentration was elevated only in SOS.

Biometric data of fish

Because of high population densities and the oligotrophic character of most high mountain lakes the growth performance of Arctic char was poor (Table 3). Stunted populations were found in Oberer Plenderlesee (OPL) and, to a lesser extent, in DR. The higher growth rate of the char populations in SOS and MPL can be explained by the decreasing population density as a result of complete reproductive failure during the last few years. In AS, however, Arctic char older than 6–7 years switched to piscivorous feeding habits, leading to continuing growth even in old individuals.

Metal concentrations in the kidney

Since no significant differences in metal concentration were found between the sexes, data from males and females were combined. Results of ANOVA and Dunn's test indicated significant differences among lakes in the metal concentration in the kidney of Arctic char (Table 5). With respect to all four metals, SOS fish only were significantly different from the fish of all other lakes. Only with respect to Cd did the fish from each lake differ significantly from each other. The concentrations of Cd and Pb in the kidney

Fig. 3. Correlation between alkalinity (ANC) and median bioconcentration factors (BCF) of metals in the kidney (dry weight) of *Salvelinus alpinus* from lakes studied. Correlation coefficients and number of fish (in parentheses) are given. Regression equations: Cd-BCF = $1.02 \times 10^6 - 2.76 \times 10^5$ log ANC; Pb-BCF = $4.24 \times 10^4 - 1.24 \times 10^4$ log ANC; Zn-BCF = $5.84 \times 10^4 + 1.64 \times 10^4$ log ANC; Cu-BCF = $2.60 \times 10^4 - 5.76 \times 10^3$ log ANC.

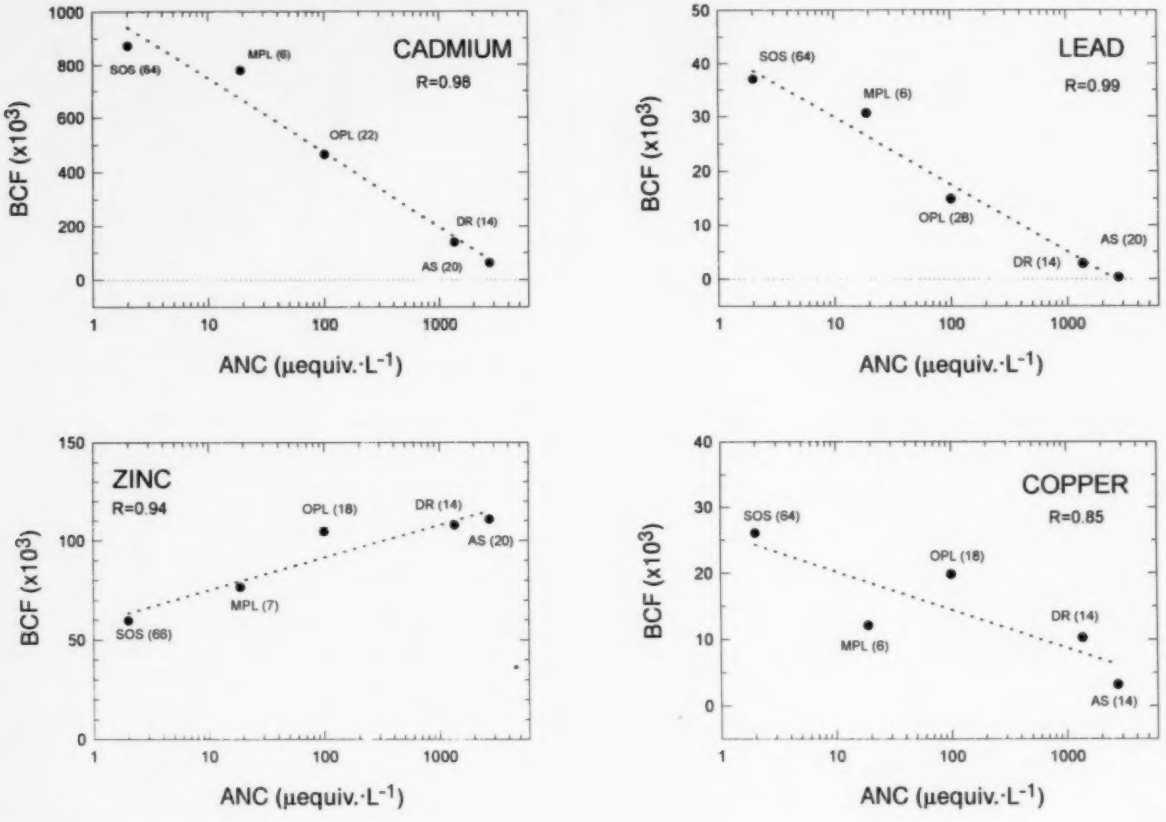


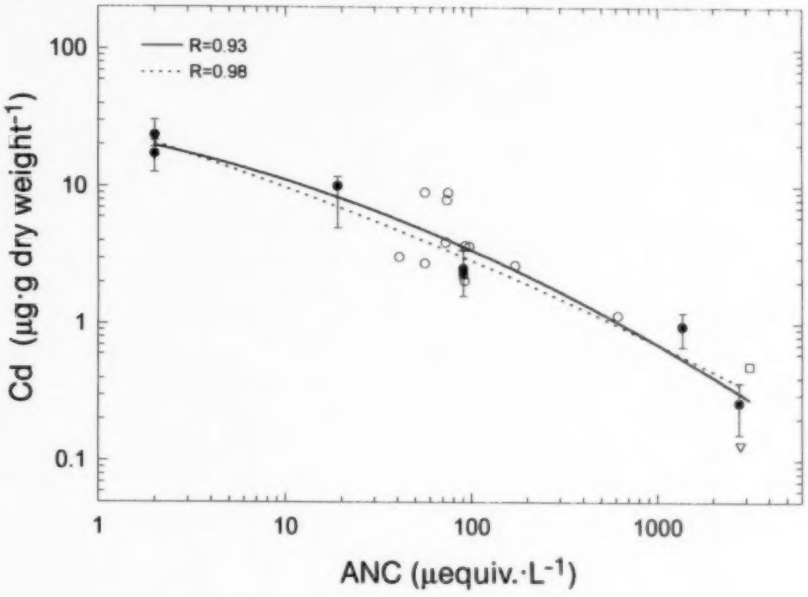
Table 5. Results of ANOVA and multiple comparisons (Dunn's test) of metal concentrations in kidneys of fish among the lakes studied.

	SOS	MPL	OPL	DR	AS
SOS		Cd, Pb, Cu, Zn	Cd, Pb, Cu, Zn	Cd, Pb, Cu, Zn	Cd, Pb, Cu, Zn
MPL			Cd	Cd, Pb, Zn	Cd, Pb, Zn
OPL				Cd, Zn	Cd, Pb, Zn
DR					Cd, Pb
AS					

Note: Only metals where significant differences occurred ($p \leq 0.05$) are shown.

of Arctic char were negatively correlated to the ANC of the lakes (Cd, $p = 0.002$; Pb, $p = 0.04$; Fig. 2). The concentration of metals in the kidney also correlated positively with the concentration of Ca (both Cd and Pb) and pH (Cd) of the lakes. Since their correlation coefficients and significance were lower than for correlations with alkalinity, results are shown only for alkalinity. In Arctic char from low-ANC lakes (OPL, MPL, SOS) the concentration of Zn was significantly higher than in fish from hardwater lakes (DR, AS). Cu, however, was elevated only in fish from the most acidic lake (SOS). In contrast to OPL, the metal concentration in fish from SOS was significantly higher in autumn than in spring (data shown only for Cd; Fig. 2). A significant positive correlation between metal concentrations in the kidney and in the water was found only for Cd ($R = 0.98$). The BCF between water and fish kidney showed metal-specific relationships with the ANC of the water (Fig. 3). The correlation with ANC was negative

Fig. 4. Concentration of Cd in the kidney of *Salvelinus alpinus* from the present study (see Fig. 2, transformed to wet weight) compared with literature data for salmonids (*Salvelinus fontinalis*, *Salmo trutta*, *Oncorhynchus mykiss*) from different oligotrophic waters. Broken line is the regression for data from the present study only, log Cd = 1.44 − 0.41 log ANC − 0.04 log ANC^2; solid line is the regression for all data, log Cd = 1.38 − 0.24 log ANC − 0.09 log ANC^2. Open symbols represent means of 4–21 fish (total number is 97). ○, data from Miller (1987); □ and ▽, G. Köck, unpublished data; ●, this study (see Fig. 3).



for Cd and Pb ($p = 0.002$), positive for Zn ($p = 0.02$), and nonsignificant for Cu.

Discussion

Dissolved Cd and Pb concentrations in the lakes studied were similar to those reported for other low-ANC lakes of varying pH (Sprenger et al. 1988; Spry and Wiener 1991) and uncontaminated waters (Wachs 1983; Merian 1991). From this point of view the extremely high BCFs in the kidney of Arctic char (up to 900×10^3 for Cd in SOS; Fig. 3) were unexpected. Cd and Pb concentrations in kidneys of char from SOS and MPL were similar to, or higher than, those in brown trout and rainbow trout from water receiving metal-polluted waste water, although concentrations of these metals in the water were considerably lower than at polluted sites (Pb in the water, 17 µg·L^{-1}; alkalinity, 2400 µequiv.·L^{-1}; Dallinger and Kautzky 1985). The Pb concentration in kidneys of fish from SOS, for instance, were similar to those in brown trout from a heavily lead-polluted river (Pb in the water; 44 µg·L^{-1}, alkalinity, 1600 µequiv.·L^{-1}; Köck et al. 1991).

Direct uptake via the gills is thought to be the major pathway of Cd and Pb uptake in freshwater fish (Merlini and Pozzi 1977*b*; Hodson et al. 1978; Pärt et al. 1985), being largely influenced by pH, alkalinity, and Ca concentrations of the water (Spry and Wiener 1991). For the vast majority of freshwater lakes a definite relationship exists between alkalinity and pH (Psenner and Catalan 1994); acidic lakes normally have low Ca concentrations. In neutral and acidic waters the free divalent ion is the dominant form of Cd and is readily absorbed by fish gills (Pärt et al. 1985; Pärt 1990). In alkaline waters, however, bioavailability of Cd is lowered by increasing adsorption, precipitation, and complexation (e.g., with bicarbonate and carbonate ligands; McCarty et al. 1978; O'Shea and Mancy 1978; Pärt et al. 1985). Competition for binding sites on the gill surface between Ca^{2+} or Mg^{2+} and divalent metal ions may decrease metal uptake in hard waters (Pagenkopf 1983; Verbost et al. 1987; Wicklund and Runn 1988; McDonald et al. 1989). Furthermore, Ca^{2+}-mediated changes in the permeability of gill membranes, leading to diminished metal accumulation in high-alkalinity waters, may influence both metal uptake and toxicity (Calamari et al. 1980; Pärt et al. 1985).

Other studies of the correlation between Cd accumulation in fish and lake pH have yielded somewhat contradictory results. Some authors reported that Cd accumulation and toxicity decrease with decreasing pH, seemingly owing to competition of H^+ ions for uptake sites on gill surface (Campbell and Stokes 1985; Pärt et al. 1985; Cusimano et al. 1986). In our study, however, Cd and Pb accumulation

correlated negatively with the pH data (not shown) and alkalinity of the lake (Figs. 2 and 3). This agrees with the results of field studies (Sprenger et al. 1988; Hamilton and Haines 1989; Stripp et al. 1990; Iivonen et al. 1992; Haines and Brumbaugh 1994) in which Cd and Pb concentrations in fish from different lakes correlated inversely with these variables.

In field studies it is not feasible to separate the effects of the two closely linked factors alkalinity and pH, but laboratory studies have shown that Cd uptake by the gills is to a large extent influenced by aqueous Ca concentration (a strong correlate of alkalinity) and is not dependent on pH over a range from 5 to 7 at a given alkalinity (Pärt et al. 1985; Wicklund and Runn 1988). Thus, it may be assumed that in the Alpine lakes studied the accumulation of Cd in Arctic char is mainly dependent on alkalinity. To assess this, we compared our data with concentrations of Cd in the kidney of salmonids (*Salvelinus fontinalis*, *Salmo trutta*, *Oncorhynchus mykiss*) from different oligotrophic waters with low waterborne Cd concentrations (≤ 0.2 μg $Cd \cdot L^{-1}$) as reported in other studies (Miller 1987; G. Köck, University of Innsbruck, Innsbruck, Austria, unpublished data; Fig. 4). Data from these studies fit very well ($R = 0.93$, $p = 0.002$) to the regression equation calculated for the data from our study ($R = 0.98$, $p < 0.01$). Although different species of salmonids were used, the similarity of the two correlations confirms the predictive power of water alkalinity for Cd concentration in the fish kidney (Fig. 4). A significant positive correlation between metal concentrations in the kidney and in the water was found only for Cd ($R = 0.98$). However, when data from the studies mentioned above were incorporated, this correlation could not be confirmed.

In contrast to Cd, water pH strongly influences the chemical speciation of Pb (Campbell and Stokes 1985; Nelson and Campbell 1991). Aqueous Pb^{2+}, which is readily absorbed by fish gills, correlates negatively with pH. In consequence, Pb accumulation increases as pH decreases (Merlini and Pozzi 1977*a*; Hodson et al. 1978; Heit et al. 1989).

Apart from pH and alkalinity the bioavailability and toxicity of metals to fish are strongly influenced by the formation of complexes with dissolved organic material in the water (Hutchinson and Sprague 1987; Campbell and Evans 1987; Spry and Wiener 1991). In general, the bioavailability of metals to fish is decreased by complexation and adsorption processes. Arctic char from our lakes showed much higher BCFs for Cd and Pb (900×10^3 and 37×10^3, respectively) than white suckers (*Catostomus commersoni*) and yellow perch (*Perca flavescens*) from Adirondack mountain lakes (BCF for kidney: 640–2140 and 40–300, respectively; recalculated on a dry weight basis from Stripp et al. 1990) with similar pH, alkalinity, and metal concentrations. Apart from possible species- and age-specific effects, differences in BCFs might be attributable to different DOC concentrations (>3.6 $mg \cdot L^{-1}$ in Adirondack lakes as against less than 1 $mg \cdot L^{-1}$ in our acidic lakes). Iivonen et al. (1992) reported a negative correlation between total organic carbon (TOC) in the water and Pb and Cd concentrations in livers of perch from 11 Finnish lakes. Furthermore, oligotrophic lakes exhibit lower sedimentation rates than more productive waters, which may lead to less effective removal of atmospherically deposited metals from the water column (Spry and Wiener 1991).

Whereas the concentrations and BCFs of Cd and Pb in the kidney of Arctic char were negatively correlated with alkalinity and pH, the patterns of Zn and Cu correlations were ambiguous in our study (Figs. 2 and 3). Some authors found no relationship between tissue Zn and lake acidity (Sprenger et al. 1988; Bendell Young and Harvey 1989; Haines and Brumbaugh 1994). Bentley (1992) demonstrated an increased Zn influx via the gills under acidic conditions, whereas Bradley and Sprague (1985) reported higher Zn accumulation rates in alkaline hard water. In our study, the concentration of Zn and the alkalinity of lake water were negatively correlated ($R = 0.99$), whereas the BCFs for Zn and water hardness were positively correlated (Fig. 3). This can be explained by additional uptake of this metal via food sources. In contrast to Cd and Pb, Zn is an essential element that can be regulated by fish over a wide range of concentrations (Sprenger et al. 1988; Bendell Young and Harvey 1989). This is also true for Cu for which higher concentrations could only be found in fish from the most acidic lake.

Dietary uptake of potentially toxic elements can be an important pathway of Cd and Pb accumulation in fish (Dallinger et al. 1987; Harrison and Klaverkamp 1989). In acidic waters both fish and benthic invertebrates contain elevated levels of metals and as the latter are important food sources they may contribute to the increased metal concentrations in fish (Czarnezki 1985; Woodward et al. 1994). Recent data of our char populations show that the Cd and Pb load of the food is considerably higher in winter (mainly chironomid larvae) than during summer (mainly allochthonous prey (Anflug); Triendl 1994). Nevertheless, we found Cd and Pb concentrations in the kidney of fish from SOS to be lowest at the end of winter and to increase during summer. Thus, we assume that gills are the primary route for Cd and Pb uptake in low-alkalinity lakes.

To ensure comparability among the lakes, only fish 6–10 years of age were analyzed in our study. Some authors have reported Cd accumulation in fish to be positively correlated with age, length, or weight (Vinikour et al. 1980; Mehrle et al. 1982; Sprenger et al. 1988). The literature on Pb is somewhat contradictory (Bohn and Fallis 1978; Wiener and Giesy 1979; Czarnezki 1985; Hamilton and Haines 1989). Statistical analysis of our data indicates a positive correlation between age of fish and Cd and Pb concentrations in the kidney ($R = 0.46$, $p = 0.005$). Because of the limited number of fish available in particular age groups it was not possible to choose an age range narrower than 6–10 years. As a consequence, variation in age may have contributed to the relatively high variation of metal concentrations in the kidney. Hammar et al. (1993) reported significantly higher levels of PCB in dwarfed Arctic char than in normally growing char. In char populations from OPL, however, no significant differences in metal concentrations between dwarfed and normally growing individuals of the same age could be detected.

Despite the considerable accumulation of Cd and Pb in Arctic char from SOS and the high toxicity potential of these metals, specimens up to 16 years old could be found. However, a high percentage of the fish older than 10 years

exhibited severe pathological changes in the liver and kidney (R. Hofer, University of Innsbruck, Innsbruck, Austria, unpublished data). Furthermore, there is evidence of reproductive failure in these fish. However, a causal correlation between high metal burden and pathological effects in the fish cannot be presently established.

In summary, analysis of the relationship between metal concentration in the kidney of fish and selected water parameters illustrates that, despite low metal concentrations in the water, the extremely high Cd and Pb concentrations in the kidney of Arctic char from acidic lakes may be attributed to the low alkalinity of the water. The good correspondence between our data and those reported from other studies confirms the predictive power of water alkalinity for Cd accumulation in fish from oligotrophic lakes.

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Effect of foraging activity on the shoal structure of cod (*Gadus morhua*)

E.M. DeBlois and G.A. Rose

Abstract: Acoustic methods used to quantify the shoaling dynamics of Atlantic cod (*Gadus morhua*) during their shoreward migration across the northeast Newfoundland shelf (spring 1992) showed that shoal structure and horizontal displacement were associated with the density distribution of small pelagic scatterers, for example, shrimp (*Pandalus borealis*). Cod speeds (to 20 km/d) were lower after encounters with shrimp (4–7 km/d). The relationship between several characteristics of the cod shoal (density, vertical spread, height off the bottom) and shrimp density was dome shaped. Internal shoal densities declined and shoal spread and height increased until shrimp densities surpassed a critical level. Above this threshold, these trends were reversed. The vertical spread of the cod shoal matched that of shrimp up to, but not beyond, a shrimp spread of 85 m. At this observed maximum in the expansion of the shoal, fish were eight body lengths apart. At all times during our survey, cod stayed within the warmer waters (>2°C) found at depths greater than 250 m. Our results are the first field verification of theoretical predictions and laboratory findings that shoals expand and contract in response to foraging activity and demonstrate the potential importance of cod aggregation dynamics to interpretations of trawl data.

Résumé : Les méthodes acoustiques, employées pour quantifier la dynamique de regroupement de la morue franche (*Gadus morhua*) pendant sa migration vers la côte en travers du nord-est du plateau de Terre-Neuve (printemps 1992), ont montré que la structure des bancs et le déplacement horizontal étaient associés à la distribution de la densité de petits éparpilleurs pélagiques, par exemple la crevette nordique (*Pandalus borealis*). La vitesse d'avancée des morues (jusqu'à 20 km/jour) baissait après les rencontres des crevettes (4–7 km/jour). La relation entre plusieurs caractéristiques du banc de morues (densité, étalement vertical, hauteur par rapport au fond) et la densité des crevettes avait une forme de dôme. La densité interne du banc baissait, tandis que son étalement et sa hauteur augmentaient jusqu'à ce que les densités de crevettes dépassent un niveau critique. Au-delà de ce seuil, les tendances étaient inversés. L'étalement vertical du banc de morues correspondait à celui du banc de crevettes, mais seulement jusqu'à une limite d'étalement de 85 m du banc de crevettes. À ce maximum observé de l'expansion du banc, l'écart entre les poissons était de huit longueurs corporelles. Tout au long de l'étude, les morues sont restées dans les eaux plus chaudes (>2°C) qui se trouvaient à des profondeurs de plus de 250 m. Nos résultats, qui sont la première vérification sur le terrain de prédictions théoriques et de résultats de laboratoire posant que les bancs se dilatent et se contractent en réaction à l'activité de recherche de nourriture, démontrent l'importance potentielle de la dynamique de l'agrégation des morues dans l'interprétation des données fournies par le chalutage.

[Traduit par la Rédaction]

Introduction

In many species, predator avoidance and foraging generate conflicting demands on time with the amount devoted to each determined, in part, by foraging rewards and perceived predation risks (Pulliam and Caraco 1984; cited in Ryer and Olla 1991). In fishes, this conflict can be partially resolved by shoaling, since fish in shoals can benefit from both improved feeding and predator avoidance (Radakov 1973; Pitcher 1986; Magurran 1990). Combining sensory capabilities among individuals increases the probability of detecting food or potential threats (Pitcher et al. 1982; Morgan 1988; Ritz 1991). Further, the patchy distribution of fish shoals decreases the probability that any one shoal will be detected by predators (Swartzman 1991). The rewards of shoaling upon food detection may include increased feeding rates as a result of decreased vigilance and social facilitation (Ranta and Kaitala 1991; Ryer and Olla 1991; Davis and Olla 1992). For fishes conducting cyclic feeding migrations, orientation precision may be improved through shoaling (Larkin and Walton 1969) and this in turn could lead to higher overall food intake among inexperienced individuals. On the other hand, fish shoals may deplete local resources more quickly than do single fish (Ranta and Kaitala 1991). Also, a tightly packed shoal leads to overlap in the visual fields of neighboring fish, which could increase competition for food (Eggers 1976;

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Morgan 1988; Robinson and Pitcher 1989; Ritz 1991; Krause et al. 1992; Krause 1993).

Several studies have linked variability in shoal structure to trade offs between the conflicting demands of feeding and predator avoidance. Shoal contraction has been demonstrated in reaction to the threat of predation; conversely, shoal expansion has been shown in response to feeding stimuli (Keenleyside 1955; Hunter 1966; Pitcher et al. 1983; Morgan 1988; Robinson and Pitcher 1989). Specifically, Keenleyside (1955) showed that threespine sticklebacks (*Gasterosteus aculeatus*) will shoal more tightly when well fed than when hungry. Hunter (1966) showed that jack mackerel (*Trachurus symmetricus*) shoals become more compact following feeding and then expand during periods of food deprivation. Morgan (1988) found that the cohesiveness of bluntnose minnow (*Pimephales notatus*) shoals increased in the presence of a predator and decreased as hunger level increased. In a study on the theoretical effect of shoaling on prey consumption, Eggers (1976) found that the behaviors described above were predictable solely on the basis of the assumption that feeding fish would minimize visual field overlap. Eggers (1976) further predicted that the cost of shoaling, or competition with nearest neighbors, should be less when prey densities were high, a factor that could lead to the contraction of the shoal beyond a certain threshold food density.

Much of the behavioral theory on fish shoaling has been derived from laboratory studies such as those cited above (also see Aoki 1984; Abrahams and Colgan 1985). Results attest to the highly plastic nature of fish aggregations. In situ, the general observations of many fisheries studies confirm the predicted plasticity of fish aggregations (e.g., Beamish 1966; Harden-Jones 1968; Aoki et al. 1986; Rose 1993; Crawford and Jorgenson 1993). However, few attempts have been made to test specific predictions of the behavioral theory on wild populations.

On the northeast Newfoundland shelf (Fig. 1), Atlantic cod (*Gadus morhua*) conduct annual postspawning migrations in spring and early summer toward inshore feeding areas (Templeman 1966; Lear and Green 1984). Rose (1993) described the specific form of these migrations; cod migrate in large cohesive aggregations (>10 km in diameter) while following physical attributes of the environment; aggregations fragment when abundant pelagic prey are encountered (typically capelin, *Mallotus villosus*, or shrimp, *Pandalus borealis*). The visual detection of pelagic food by cod has also been described by Brawn (1969). In 1992, a large cod aggregation was tracked over 36 d with scientific echosounders during its feeding migrations across the northeast Newfoundland shelf (Rose 1993). During tracking, detailed data on cod shoal structure and pelagic prey fields along the migratory route were collected.

In the present study, we test behavioral predictions arising from theory and laboratory studies on the effects of prey densities and foraging behaviors on the shoal structure of free-ranging Atlantic cod. We evaluate the prediction that the spatial structure of migrating and feeding cod shoals will be affected by prey densities encountered along the route. More specifically, we test the hypothesis that cod shoals will expand in the presence of increasing prey densities, but contract if prey densities surpass some threshold.

Methods

Survey area and acoustic sampling

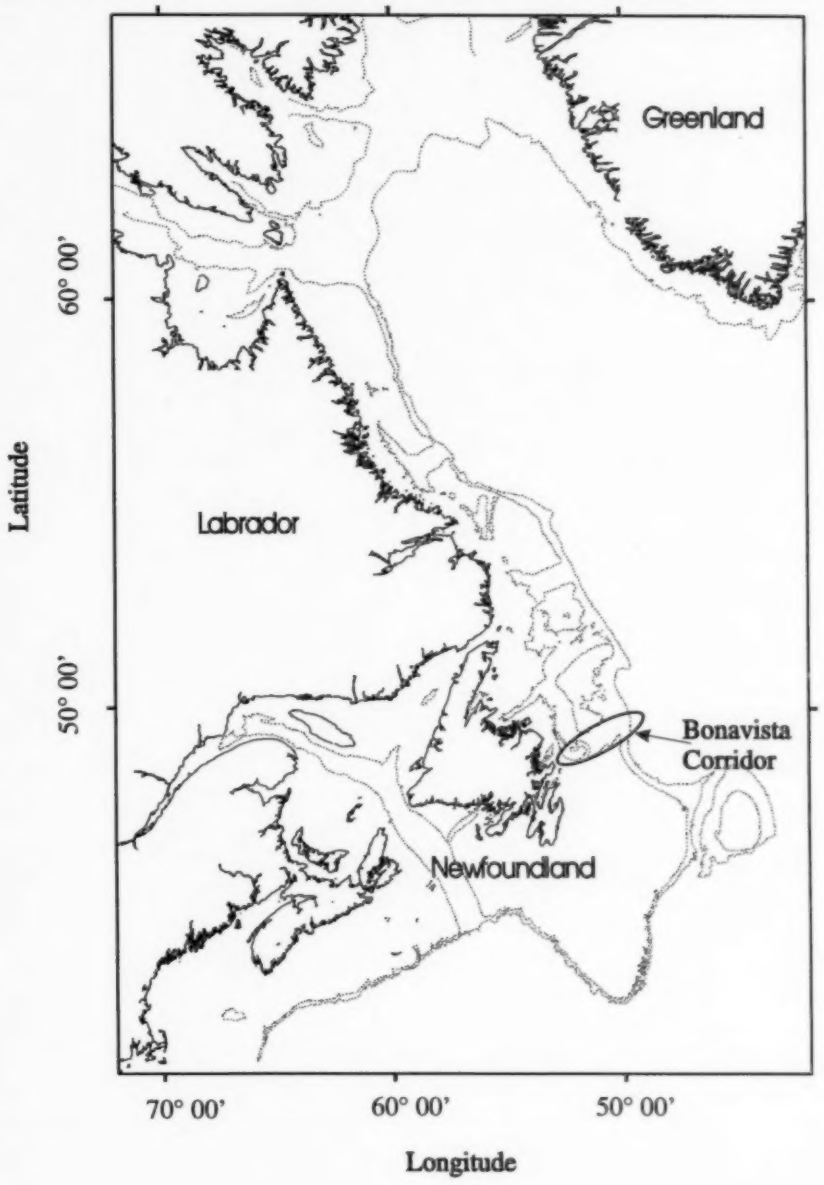
A large shoal of spawning cod was located within the outer reaches of the Bonavista migration corridor on June 8, 1992 (Rose 1993) (Fig. 1). This aggregation was later joined by juvenile cod and the inshore migration across the northeast Newfoundland shelf commenced. These cod were tracked for 36 d (June 8 – July 14) with two echosounder-equipped vessels as they migrated across the northeast Newfoundland Shelf within the Bonavista "highway" (Rose 1993) (no data were collected on June 20–24 as ships were changed). Both the RV *Gadus Atlantica* and the RV *Petrel V* were equipped with 38-kHz dual beam echosounders (Biosonics Inc). Cod are unlikely to be aware of frequencies above 2 kHz (Schwarz 1985). Boat avoidance was also unlikely at these depths (ca. 300 m) (Ona and Godo 1990; G.A. Rose, unpublished data).

The echosounding systems were calibrated (SE <0.1 dB) with a tungsten–carbide standard reference sphere both before and after the tracking, according to methods adapted from MacLennan and Simmonds (1991). The transect strategy was to criss-cross the aggregation continuously, attempting to transect the centre, define the aggregation edge, and map the surrounding area. From 5 to 18 transects were run daily (mean 8 ± 1.4) across and around the shoal so that nearly continuous contact was maintained. After the ship change, the shoal was in the same location and had the same dimensions as when left and no cod were located further seaward in the migration track. Hence, the probability that this shoal was not the same as that tracked during the first phase of the tracking was considered minimal.

Cod and small pelagic scatterers were separated by combining target strength data, echogram interpretations (e.g., Fig. 2), and fishing set data. The mean in situ target strength of the small pelagic scatterers was −65 dB, well below that of cod (−34 dB) or other fishes potentially common in this area (G.A. Rose, unpublished data). In this paper we will refer to the small pelagic scatterers as shrimp, although other invertebrates (e.g., euphasids) were almost certainly a component of this signal. The designation is based on bottom and midwater trawl fishing set data (with small mesh liners) that indicated shrimp (*Pandalus borealis*) was the dominant species within this size-class and stomach content data that indicated that shrimp were the main prey of cod during this study.

To support the acoustic methods, 55 fishing sets were conducted from the RV *Gadus Atlantica* and the RV *Lady Hammond* with midwater (Diamond IX) and bottom trawls (Engels 145 high-rise and Western IIA). Forty-five of these sets were directed at cod, 10 at the small pelagic scatterers. All sets directed at cod caught cod (mean catch per 30-min set was 1000 fish, maximum 5000) and most also caught some shrimp. Mean cod length was 46 ± 0.5 cm (±95% CIs). All sets directed at small pelagic scatterers caught shrimp (maximum catch 10 kg; no further quantification was attempted for shrimp because of uncertainties associated with our fishing methods). Following taxonomic classification, standard echo integration methods were used to calculate the relative acoustic densities of cod and shrimp over a grid with horizontal and vertical dimensions of

Fig. 1. Northeast Newfoundland shelf (Canada). Acoustic and trawl samples were collected within the Bonavista migration corridor described by Rose (1993).



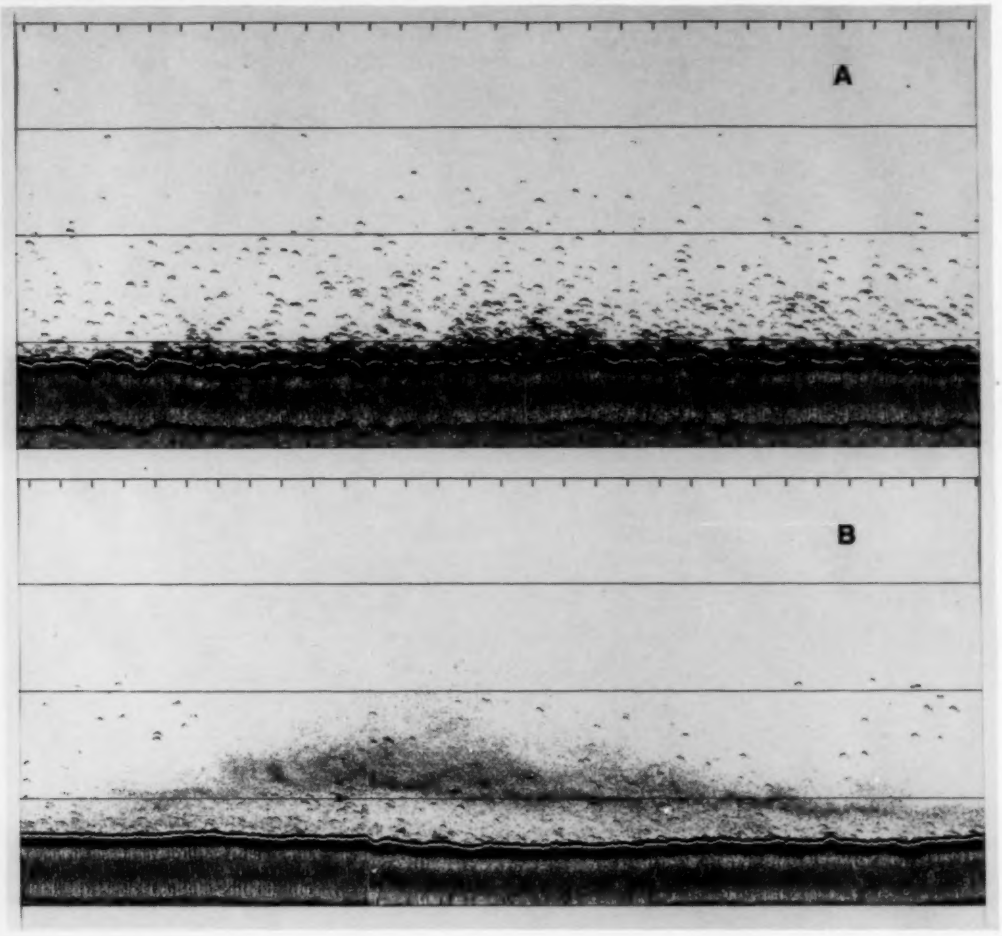
1000 or 100 × 10 m (MacLennan and Simmonds 1991). Cod densities were scaled to absolute values with an estimate of mean target strength per fish (−34 dB) based on in situ and ex situ experimentation (G.A. Rose, unpublished data). A scaling factor of −65 dB was used for shrimp. However, because of the uncertainty of this estimate and of the proportion of the full biomass measured at 38 kHz, the estimated density levels for shrimp should be interpreted as relative values.

Cod distribution and prey availability

Daily cod centroids were calculated as the mean latitude and longitude of cod positions weighted by acoustic densities. On the basis of these positions, the lateral movements of cod were compared with the distribution of available food over the sampled area and with the amount of food consumed as defined by stomach content.

Cod stomachs were collected from all fishing sets (see above) from June 9 to July 14 at intervals of 2 ± 0.62 d

Fig. 2. Echograms of cod (A) and shrimp (B) along the Bonavista corridor in the spring of 1992. Total depth in each panel is 325–350 m (only bottom waters are shown). Total horizontal view is 4.3 km. Individual cod can be seen as inverted Vs in both A and B. Shrimp are lighter specks prominent in B.



with a 2-week gap occurring from June 17 to July 1. Stomach data from multiple sets taken on any given day were averaged to provide a total of 14 daily estimates of cod consumption each based on an average of 64 ± 29 cod. On days when trawl data were collected, one to nine trawling sets were made in conjunction with acoustic sampling. A stratified-random sample of three fish per 9-cm length group was taken from the catch of every set. Cod stomachs were processed and total gut fullness was calculated as in Lilly (1991), as follows:

$$\mathrm{TFI} = \sum_{i=1}^{m} \mathrm{PFI} = 1/n \sum_{j=1}^{n} W_{ij}/(L_j)^3 \times 10^4$$

where TFI is total gut fullness, m is the number of prey categories, PFI is partial gut fullness calculated for each prey category i, W is the weight (grams) of prey i in fish j, L is fish length (centimetres), and n is the number of fish in any sample.

This index can be used as an estimate of feeding rate (grams per cubic centimetre) (see Lilly 1991). However, owing to the small number of estimates of TFI available (14 daily estimates), we used acoustic estimates of prey density to assess the proclivity of cod toward feeding and largely restricted our use of the above feeding indices to a general taxonomic description of prey consumed.

Shoal structure

To describe daily variability in the shoal structure of cod, we averaged information from all acoustic transects made within 24 h (00:00–24:00). The variables shoal density, vertical spread, and height were used to characterize shoal structure. Shoal density was defined as the number of cod per cubic metre averaged over shoal breadth and depth. To normalize the observed frequency distribution, density values were transformed to their natural logarithms prior to analyses. Shoal spread and height from the bottom were derived

Fig. 3. Diagram of shoal height and vertical spread. Spread is the vertical distance (m) between the 5th and the 95th percentiles in cod density. Height off the bottom is the distance (m) between the bottom and the 50th percentile in cod density.

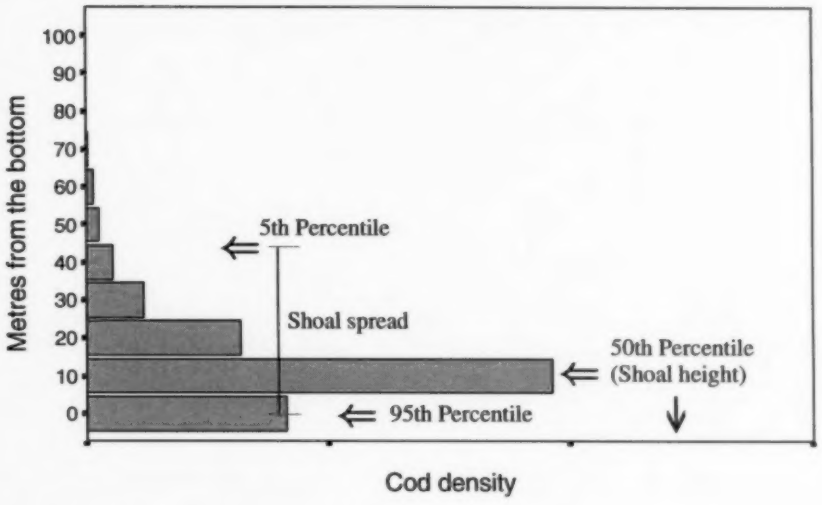
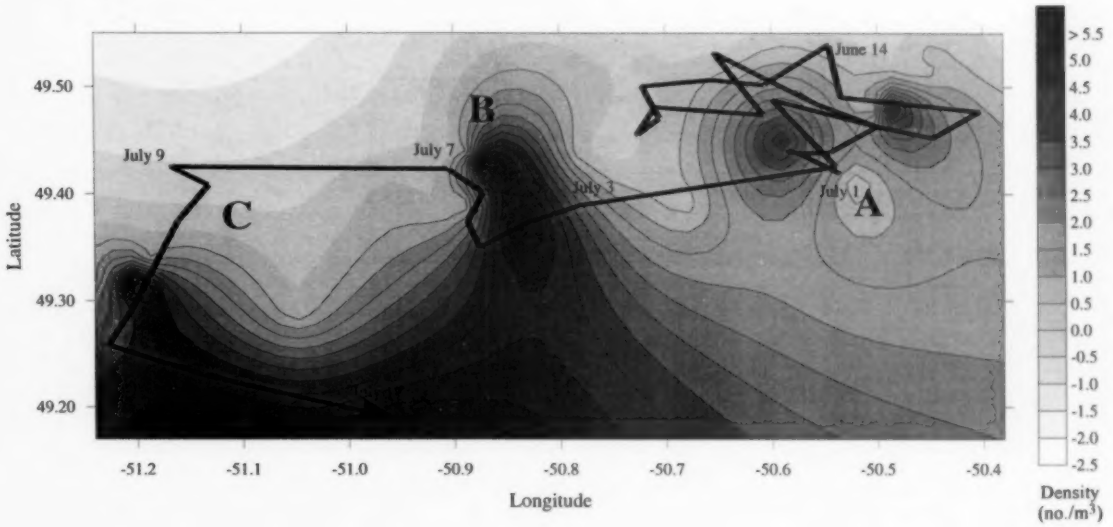


Fig. 4. Time-integrated summary of shrimp density (June 8 – July 14, 1992). Cod movement is shown as a black arrow. High speeds (≥20 km/d) were noted from A to B and from B to C. Shrimp density contours were generated by normal kriging (Surfer version 5.01; Golden Software Inc.).



from the depth structure of untransformed density measurements collapsed to a vertical plane. On any given day, the distribution of these densities was unimodal and log-normal over depth. Shoal spread was defined as the vertical distance (metres) between the location of 5th and 95th percentiles in cod density. The height of the cod shoal off the bottom was calculated as the distance between the bottom and the location of the 50th percentile in cod density (Fig. 3).

To describe behavioral changes accompanying feeding activity, estimates of shoal density, spread, and height were reduced to one dimension using principal component analysis (PCA) (see Lebart et al. 1984). The relationships between these PCA scores and shrimp density (number per cubic metre), spread, and height off the bottom (calculated as above) were described using least-squares regression (Sokal and Rohlf 1981). Values of shrimp spread were

Fig. 5. Cod diet from June 9 to July 14, 1992. PFI values (see text) for prey other than the three dominant forms did not exceed 0.20.

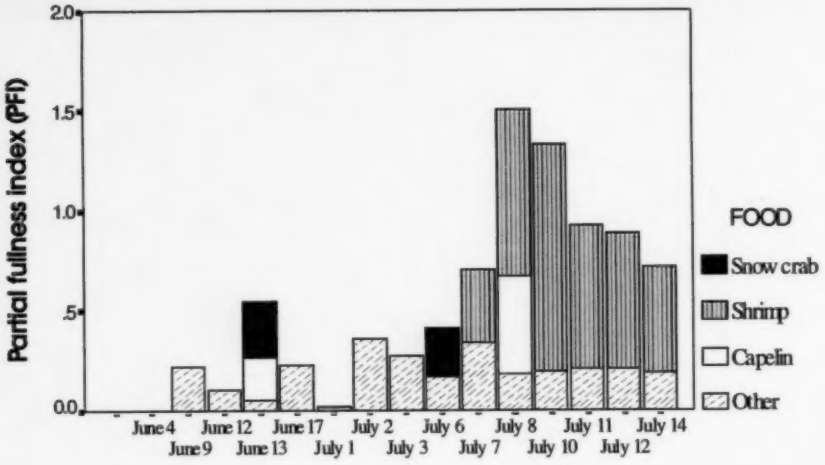
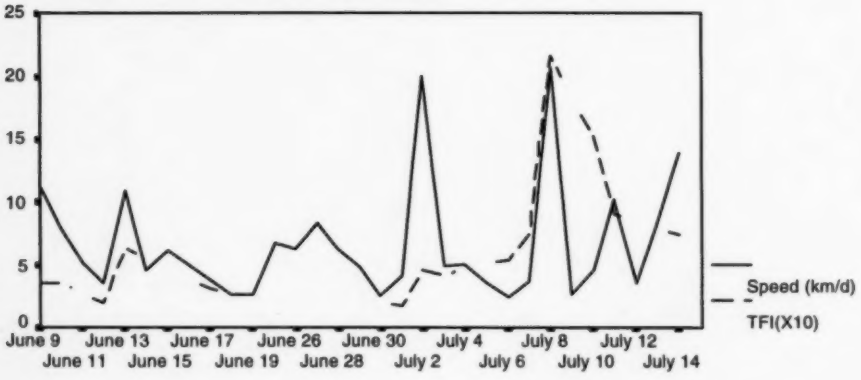


Fig. 6. Daily variation in cod speed (km/d) and gut fullness (TFI, ×10).



generated from June 24 to July 14 only. Given acoustic data resolved to 10-m depth strata (see above), spread calculations required animals to be present within at least two 10-m depth layers. This condition was not satisfied for shrimp prior to June 24.

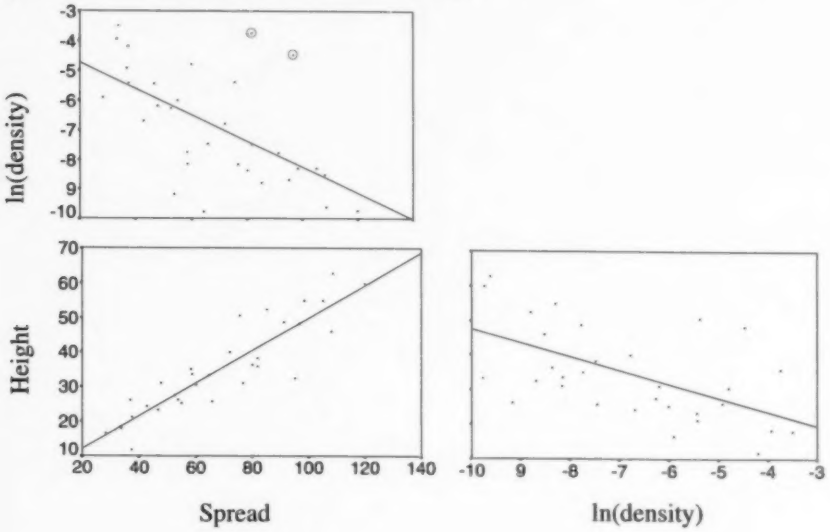
Finally, to assess if the expansion and contraction of the shoal was more or less uniform both vertically and horizontally, we generated an indirect measure of change in horizontal spread for the cod shoal using the relationship between vertical spread and density. We refer here to horizontal expansion relative to change in vertical spread rather than to the absolute size of the shoal. Our calculations assumed that the expansion of a shoal with constant fish numbers would lead to a predictable decline in the number of fish measured per unit area within the boundaries of that shoal. Through a series of simulations in which we assumed that the change in horizontal spread would be 0 to 2 times that of vertical spread, we defined the expected relationship between ln(vertical spread) and ln(density). More particularly, we determined that the slope of the ln(vertical spread) to ln(density) relationship was highly sensitive to the ratio between changes in horizontal and vertical spread. Using field data, we were then able to compare the observed slope between ln(vertical spread) and ln(density) with our predictions and generate an estimate of corresponding changes in horizontal spread in situ. We caution, however, that since our predictions relied on the simplifying assumption that the shoal expands and contracts as a homogeneous rectangle, our estimate of change in horizontal spread should be seen as a coarse approximation.

Results

Cod distribution and prey availability

From June 8 to July 14, 1992, the vertical expansion of cod and shrimp exhibited a persistent overlap. On average, the cod shoal extended from 3 to 65 m off the bottom whereas shrimp spread from 14 m off the bottom up to 100 m (e.g., Fig. 2). Laterally, cod moved in starts and

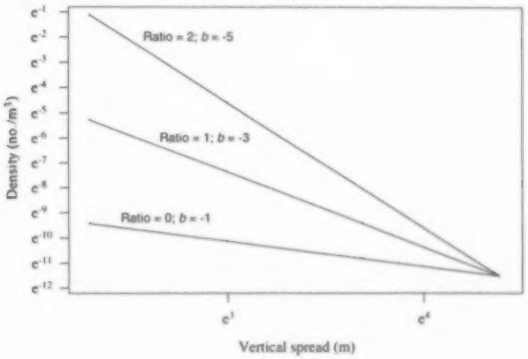
Fig. 7. Correlation between the variables vertical spread (m), ln(density (no./m^3)), and height off the bottom (m). All correlations were significant at $p < 0.001$. Note that in the regression of ln(density) versus ln(spread) (see text), circled outliers were removed to generate a more representative estimate of the slope.



stops and at times retreated seaward as they migrated shoreward along the Bonavista migration corridor (see Rose 1993 for additional detail). In general, stops and retreats coincided with areas of elevated densities of shrimp. Through June, during their period of most intense spawning (>90% females spent by July 1; G.A. Rose, unpublished data), cod movements were characterized by little shoreward and low daily displacements and speeds (mean daily speed 6 km/d) (see area A in Fig. 4). Cod did feed during spawning (Fig. 5, June 13) even though mean (geometric) shrimp densities were much lower during June (0.0003/m^3) than afterward (0.18/m^3) ($t = 6.08$, $p < 0.0001$). On July 1, the cod aggregation moved quickly shoreward (20 km/d) out of the spawning area and then stopped in an area having elevated shrimp densities. Cod remained in this area of abundant shrimp for 5 d, moving slowly at a rate of 4 km/d. Following this slowdown, on July 7, cod again moved quickly at 21 km/d (areas B and C in Fig. 4), then progressed more slowly (7 km/d) over an increasing gradient in shrimp density. We noted some association between the mean speed of the cod shoal and the average gut fullness of individuals within the shoal (Fig. 6). In particular, each peak in TFI matched a peak in speed and was followed by a sharp decline in speed and distance travelled. These data were judged to be too few for further statistical analysis.

Cod diet was dominated by shrimp (*Pandalus borealis*) (mean of stomach content 51.3%). Snow crab (*Chionoecetes opilio*) and capelin (*M. villosus*) accounted for much smaller proportions, 17.3 and 16.6%, respectively, of cod diet and were important only at the beginning of our sampling interval (May 31 – July 6) (Fig. 5). Cod fed almost exclusively on shrimp from July 6 to 14 (Fig. 5). Feeding rates over this later part of the survey were 3 times higher than they were

Fig. 8. Predicted relationship between vertical spread (m) and density (no./m^3) of cod shoal. Expected slopes (b) are given for ratios of horizontal to vertical spread ranging from zero to two. The general relationship between these ratios (R) and the slope (b) of ln(vertical spread) versus ln(density) is $R = -0.5 - 0.5b$.



at the beginning ($t_{11} = 3.07$, $p = 0.01$), increasing from 0.37 ± 0.12 g/cm^3 prior to July 6 to an average of 1.11 ± 0.49 g/cm^3 thereafter. Shrimp were present over much of the sampled area and in the acoustic and trawl records. Capelin were not. No data exist on snow crab distribution. Hence, this analysis deals solely with cod responses to availability of *Pandalus borealis*.

Shoal structure

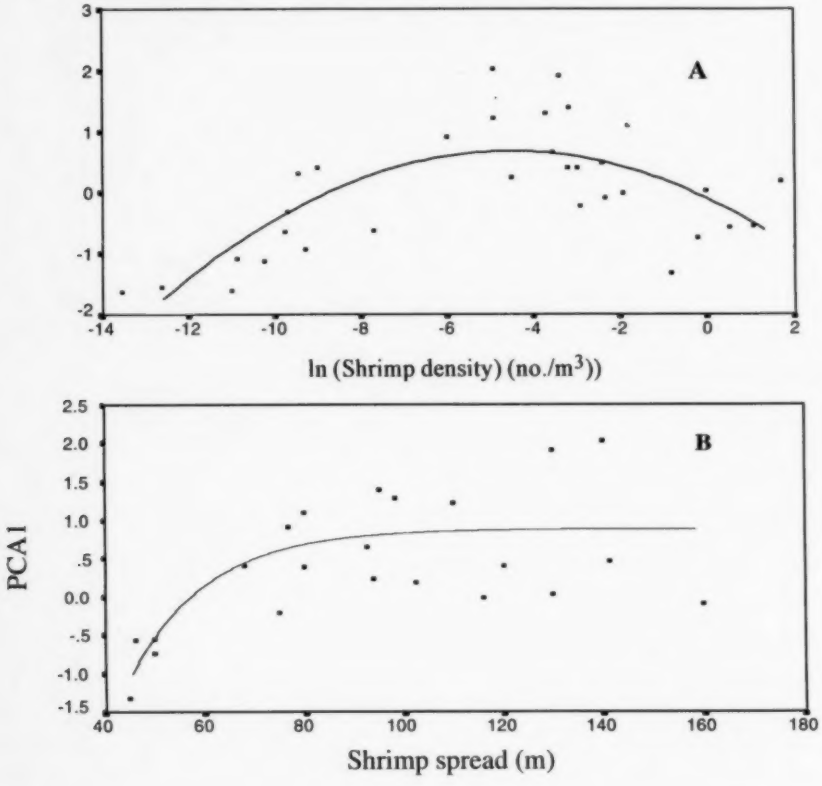
Estimates of the vertical spread of the cod shoal, its height off the bottom, and ln(cod density) were strongly collinear

Table 1. Statistics from principal components analysis on vertical spread, ln(density), and shoal height as well as factor loadings for PCA axis 1.

PCA axis	Eigenvalue	% of variance explained	Factor loadings		
			Vertical spread	ln(density)	Height
1	2.39	80	0.95	0.79	0.93
2	0.52	17			
3	0.10	3			

Note: Vertical spread and height loaded positively whereas ln(density) loaded negatively.

Fig. 9. Relationship between PCA1 scores and (A) shrimp density (I) (PCA1 = $-0.09 - 0.34(\ln I) - 0.04(\ln I)^2$; $F_{[2,29]} = 17.43$, $p < 0.0001$, $R^2 = 0.55$) and (B) shrimp spread (IS) (PCA1 = $0.82 - 30.93\ (e^{-0.06(IS)})$; $F_{[3,18]} = 9.27$, $p < 0.05$, $R^2 = 0.51$).



(Fig. 7). Increases in vertical spread were reflected in the horizontal distribution of the shoal. The relationship between ln(vertical spread) and ln(density) yields a slope of -3.5 ± 1.0 ($p_{slope} < 0.0001$, $r^2_{model} = 0.58$), which according to predicted values (see Fig. 8) indicates a ratio of 1.25 between change in horizontal spread relative to vertical spread. We do not, however, use horizontal spread in subsequent analyses, since as noted previously we could only approximate its general relationship to variability in vertical spread.

From PCA, we found that PCA axis 1 (PCA1) could summarize 80% of the variability in vertical spread, ln(density), and the height of the shoal off the bottom (Table 1). Concisely, low PCA1 scores indicate a high-density, low-spread shoal located close to the bottom. High scores indicate a low-density, high-spread aggregation, located further up in the water column. PCA1 scores showed a domed relationship to shrimp density (I), following PCA1 = $-0.09 - 0.34(\ln I) - 0.04(\ln I)^2$ ($F_{[2,29]} = 17.43$, $p < 0.0001$, $R^2 = 0.55$; Fig. 9*a*). When plotted against shrimp spread (IS), PCA1 scores increased to asymptotic values at and above shrimp spreads of ca. 85 m, following PCA1 = $0.82 - 30.93(e^{-0.06(IS)})$ ($F_{[3,18]} = 9.27$, $p < 0.05$, $R^2 = 0.51$; Fig. 9*b*). No relationship was noted between PCA1 scores and the height of the shrimp swarm.

Regression equations summarizing the relationship between the original variables (Table 2) basically confirmed relationships obtained with PCA1 scores. Of particular interest, the slope of the regression line between cod spread and shrimp spread, at shrimp spread < 85 m (1.16 ± 0.57), did not differ from 1 ($t_8 = 0.13$, $p < 0.0005$). At shrimp spreads > 85 m, cod spread remained relatively constant at 86 m and shoal height and density remained stable at 43 m off bottom and 0.0002 cod/m^3.

Discussion

Our data indicate that during the migratory period under study, and within the larger scale confines of the Bonavista migration corridor (Rose 1993) and its physical structures, the horizontal displacement of cod showed a remarkable association with the density distribution of the chief prey encountered, in this case small pelagic scatterers likely dominated by shrimp. The horizontal and vertical distribution of cod and these shrimp overlapped, providing ample foraging opportunity for cod. In addition, changing cod speeds suggested that the shoal reduced its speed by a factor of three to five (20 vs. 4–7 km/d) shortly after entering abundant shrimp patches. This finding agrees with in situ experiments by Jones (1978) who noted high swimming speeds for cod before food encounter and low speeds upon encounter and with results from Björnsson (1993) which showed that the mean swimming speed for cod tested in aquaria was highest (18 km/d) when search for food would be intense (food density = one-half maximum intake) and lowest (6 km/d) when food was unlimited or absent. Cod gut fullness levels noted in this study (TFI = 0.71 ± 0.16) were within the expected range for cod feeding on the Newfoundland shelf. Maximal gut fullness occurs at a TFI of ca. 12 (Lilly 1991).

Our data demonstrate that cod shoaling behavior was closely related to foraging activity. Cod spread out and moved up into the water column on encountering increasing shrimp densities, and we assume increasing foraging opportunity. This dispersive trend was maintained up to a critical prey density, D_{crit}. Beyond D_{crit}, the trend in cod behavior was reversed and further increases in shrimp density led to a gradual contraction of the cod shoal. We were unable to estimate the absolute value of D_{crit} without a better estimate of target strength for *Pandulus borealis* and assurance that integrated densities (38 kHz) represent the full invertebrate biomass present. However, the relative values used in our study confirm the presence of a domed relationship between cod shoaling parameters and shrimp density. In reaction to increasing shrimp spread, cod spread out and up into the water column to a maximal vertical spread of 86 m and a height of 43 m off the bottom. Our calculations indicate that changes in horizontal shoal spread were roughly proportional to changes in vertical spread.

Our study provides the first field evidence that fish shoals expand and contract in reaction to food and foraging (Keenleyside 1955; Eggers 1976; Pitcher et al. 1983; Morgan 1988; Robinson and Pitcher 1989). Shoal expansion in response to feeding activity is thought to reduce the overlap in the visual field of neighboring fish, thereby decreasing competition (Eggers 1976; Morgan 1988; Robinson and Pitcher 1989). At high prey density, predator–prey encounter rates could be sufficiently high to allow effective foraging in a more compact shoal. Theoretical studies, for example, those by Larkin and Walton (1969) and Swartzman (1991), have suggested that compact shoaling is a more effective predator avoidance behavior and allows for increased migratory precision.

Table 2. Regression equations summarizing the relationships between ln(cod density (no./m^3)) (D), vertical spread in metres (S), and shoal height (H) off the bottom against both ln(shrimp density (no./cm^{-3})) and shrimp spread.

y	a	b	c	F	p	R^2
		$y = a + b(\ln I) + c(\ln I)^2$				
$\ln(D)$	−7.46	0.36	0.06	28.31	0.0001	0.64
S	60.18	−10.01	−0.98	11.75	0.0002	0.41
H	3.78	−4.10	−0.42	7.66	0.0021	0.30
		$y = a - b(e^{-cIS})$				
$\ln(D)$	−8.78	−16.90	0.04	807.14	<0.0001	0.61
S	86	1256	0.07	114.59	<0.0001	0.46
H	43	370	0.06	87.11	<0.0001	0.32

Note: Relationships with shrimp density ($\ln I$) followed the form $a + b(\ln I) + c(\ln I)^2$ whereas relationships with shrimp spread (IS) followed $a - b(e^{-cIS})$. For the F test for shrimp density, df = 2,29; for shrimp spread, df = 3,18.

The vertical spread of the cod shoal matched that of its forage patch up to a shrimp spread of 85 m. Little is known about the influence of prey patch area on predator distribution within a patch. Foraging studies have focussed on predator distribution and selection among patches (e.g., van Durne and Glass 1992; Krause 1992). However, Morgan (1988) speculated that food patch area might affect the cohesiveness of a foraging shoal of bluntnose minnow (*Pimephales notatus*) and that the spread of fish shoals relative to patch area could increase in response to interference competition.

Beyond a shrimp spread of 85 m, we noted little increase in the vertical spread or height of the cod shoal. Cod may not have extended their distribution beyond 85 m because of a decrease in competition mediated by visual distance as cod spread upward and densities declined. From a relationship developed with saithe (*Pollachius virens*) between nearest neighbor distance and fish density (Fig. 5 in Pitcher and Partridge 1979), we calculated that when shrimp were spread over their observed minimum of 45 m, cod were foraging at a distance of four body lengths from their nearest neighbors. At a shrimp spread of 85 m and beyond, nearest neighbor distance increased to a maximum of eight body lengths. This maximum value is very similar to the estimated maximum dispersion of cod during migration and attributed to the maximum distance over which cod could see one another in these waters (Rose 1993). Perhaps this visual limit sets the density and shoal height limit that can be expected from migrating and foraging cod. Conversely, both these values are much higher than the estimated nearest neighbor distance of one body length noted while cod were spawning (June 8–19) (Rose 1993).

An alternative explanation for the maxima in shoal spread and height could lie in the thermal structure of the water column in the area: cold shelf waters overlie warmer oceanic waters (Rose 1993). A shoal spread of 86 m coupled with a maximum height off the bottom of 43 m effectively restricted the cod shoal to depths of 260–350 m, corresponding to a temperature range of 2.2–2.8°C. Rose (1993) noted that cod tend to follow warm-water channels ranging in temperature between 2 and 2.5°C during their annual spring migration across the northeast Newfoundland shelf. Data presented in other studies report that cod are typically found in rather stable thermal ranges (Nakken and Raknes 1987; Wells et al. 1988; Rose and Leggett 1988, 1989; Rose et al. 1994). Although cod have been observed to make foraging forays to waters having temperatures well outside their current range (Rose and Leggett 1989, 1990; G.A. Rose, unpublished data), it is still conceivable that temperature limited cod movement toward shrimp. In the Rose and Leggett (1989, 1990) studies, the prey, in this case capelin, occupied a thermal habitat fully separate from that of cod. To feed, cod needed to swim through, and readily swam through, waters having temperatures they otherwise did not occupy. In the present study, the vertical distribution of shrimp spanned that of cod and included the warmer waters preferred by cod during their shoreward migration. Hence, in this case, prey were available both where temperatures were favourable and where they were not. Given this choice, cod may have restricted the range of their feeding on shrimp to maintain stable temperatures.

Our results focussed on cod predation on shrimp but we believe they may apply to other prey. Although *Pandulus borealis* is a known forage species for cod in our study area, capelin often constitutes the larger part of cod diet in this region (see Lilly and Parsons 1991). We do have indirect evidence that the behaviors noted in this study may hold for capelin. Lilly (1994) found a negative relationship between the size of cod catches obtained in bottom trawl surveys and the amount of capelin ingested by these cod. Bottom trawls are less effective on dispersed fish than on shoals aggregated close to the bottom. If cod react to capelin as they did to shrimp, then cod would disperse and expand into the water column while foraging and contract toward the bottom when capelin are not available. In bottom trawl samples, these behaviors would translate into low catches of well-fed cod and high catches of cod with little in their gut, as observed. It follows that the interpretation of fish distribution and feeding data from trawl surveys are likely to be strongly biased by variability in shoal structure caused by interactions between predators and their prey.

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Postsettlement eutrophication histories of six British Columbia (Canada) lakes

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Abstract: Eutrophication is a serious problem in many British Columbia lakes. However, long-term nutrient data are rare or unavailable for most lake systems, so the natural, predisturbance characteristics of lakes are unknown, as are the trajectories of past environmental change. We used paleolimnological analyses of diatoms to quantitatively assess eutrophication trends for approximately the last 150 years in six British Columbia lakes. A transfer function was used to infer past lake-water total phosphorus concentrations from the sedimentary diatom assemblages in ^{210}Pb-dated sediment cores: all of the lakes had relatively high total phosphorus levels (>13 μg/L) prior to European settlement. Three of the lakes showed significant eutrophication since that time, whereas the others were only mildly affected. Total phosphorus inferences using the transfer function satisfactorily estimated the modern total phosphorus concentrations of our six study lakes. Minor quantitative problems arose when some fossil assemblages provided poor analogues to the calibration function, but eutrophication trends were still clearly apparent. Our results confirm that some British Columbia lakes have suffered considerable eutrophication as a result of anthropogenically related nutrient inputs, while others, although situated within human-influenced regions, have been relatively unaffected. These results can now be used to help set realistic goals for restoration projects.

Résumé : L'eutrophisation pose un grave problème dans de nombreux lacs de Colombie-Britannique. Toutefois, pour la plupart des systèmes lacustres, on ne dispose dans les meilleurs des cas que de rares données à long terme sur les matières nutritives, de sorte qu'on ne connaît pas les caractéristiques naturelles des lacs avant perturbation, pas plus que l'histoire des modifications antérieures de l'environnement. Nous avons eu recours à des analyses paléolimnologiques des diatomées pour évaluer de façon quantitative les tendances de l'eutrophisation, pendant les 150 dernières années environ, dans six lacs de la province. Une fonction de transfert a servi à reconstituer les concentrations passées de phosphore total dans l'eau des lacs à partir des assemblages de diatomées sédimentaires dans des carottes de sédiments datées au ^{210}Pb : tous les lacs présentaient des niveaux relativement élevés de phosphore total (>13 μg/L) avant la colonisation européenne. Trois des lacs ont connu par la suite une eutrophisation notable, tandis que les autres n'ont été que légèrement touchés. Le calcul des concentrations de phosphore total à l'aide de la fonction de transfert a permis d'estimer de façon satisfaisante les concentrations actuelles de phosphore total dans les six lacs de l'étude. Des problèmes quantitatifs mineurs se sont posés quand certains assemblages fossiles n'ont fourni que des analogues médiocres à la fonction d'étalonnage, mais les tendances à l'eutrophisation restaient nettement apparentes. Nos résultats confirment que certains lacs de Colombie-Britannique ont subi une eutrophisation considérable à la suite d'apports anthropiques de matières nutritives, tandis que d'autres, quoique situés dans des régions soumises à l'influence humaine, ont été relativement peu atteints. Ces résultats peuvent maintenant servir à fixer des objectifs réalistes pour les projets de rétablissement.

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Introduction

British Columbia (B.C.) contains many lakes that are believed to have become eutrophic as a result of human activities since European settlement, circa 1850 (Northcote and Larkin 1963; Stockner and Northcote 1974). Possible nutrient sources have included direct inputs (such as sewage disposal) and indirect sources (such as runoff from pastures, and more recently, agricultural fertilizers). Deterioration of water quality, together with increased agricultural and industrial water demands, have jeopardized aspects of the B.C. economy that are based on tourism and recreational activities. Lake restoration measures are therefore desirable, but it is first necessary to determine the causes and timing of lake-water quality deterioration. Unfortunately, as is typically the case with water quality assessments (Smol 1992, 1995), most lake monitoring programs in B.C. did not begin until a problem had been identified. Using paleolimnology, we can identify trophic changes that occurred before conventional monitoring programs were initiated (Charles et al. 1994).

Paleolimnology is the synthetic science that is used to describe the physical, chemical, and biological histories of aquatic systems. This is achieved by analyzing materials archived in the sedimentary record. Attempts at reconstructing past nutrient concentrations by simply analyzing the phosphorus content of sediments are unreliable, as sedimentary phosphorus concentrations are affected by many factors, such as redox conditions and concentrations of coprecipitates (Engstrom and Wright 1984). Instead, proxy methods, such as those using past diatom species composition, must be used (Anderson and Rippey 1994).

Paleolimnological analyses aimed at reconstructing past eutrophication trends have been conducted in many regions (Anderson et al. 1993; Fritz et al. 1993; Salonen et al. 1993; Brugam 1988; Stoermer et al. 1985), including a few in B.C. (e.g., Stockner and Northcote 1974). Sedimented diatoms are sensitive indicators of nutrient concentrations and can be used to infer past water quality. The remains of diatoms have been considered the most useful paleoindicators for several reasons: their siliceous cell walls make them resistant to dissolution and breakage, they occur in most environments where water is present, they are very diverse and often identifiable to the level of species or subspecies, and their taxonomy is generally well described (Dixit et al. 1992). Stockner and Northcote (1974) used diatoms in a pioneering study to reconstruct the trophic histories of four B.C. lakes, but their methods were based on the qualitative environmental preferences of a few common diatom genera. Paleolimnologists can now use more advanced inference methods to infer past lake-water quality.

It is possible to estimate quantitatively the relationship of a taxon to a given environmental variable, and to use such relationships to infer that variable from fossil diatom assemblages (Charles and Smol 1994). These relationships are obtained by assessing the diatom assemblages from the surficial sediments of several lakes and relating the species distributions to modern limnological conditions. Several diatom-based transfer functions have been developed to infer environmental characteristics from diatom assemblage data. For example, pH (Birks et al. 1990), salinity (Cumming and Smol 1993), and nutrient (e.g., Agbeti 1992; Anderson et al. 1993; Hall and Smol 1992) inference functions have been constructed using weighted-averaging regression and calibration approaches. This method assumes that diatom species respond in a unimodal fashion to environmental variables, and thus an environmental optimum can be determined for each taxon. A quantitative estimate for a fossil sample is then determined from the average of the optima of all taxa in that sample, weighted by their relative abundances.

The main objective of this paper was to infer past trends in total phosphorus concentration ([TP]) for one presently mesotrophic and five eutrophic B.C. lakes. To gain a historical perspective on eutrophication in B.C., past changes in [TP] were inferred from diatom valves in lake sediment cores from the region. We used a [TP] inference model that was originally designed by Hall and Smol (1992) and has been recently updated (Reavie et al. 1995) to increase the range of [TP] optima represented by the diatom species. We also evaluate the reconstructive ability of this new model.

Site descriptions

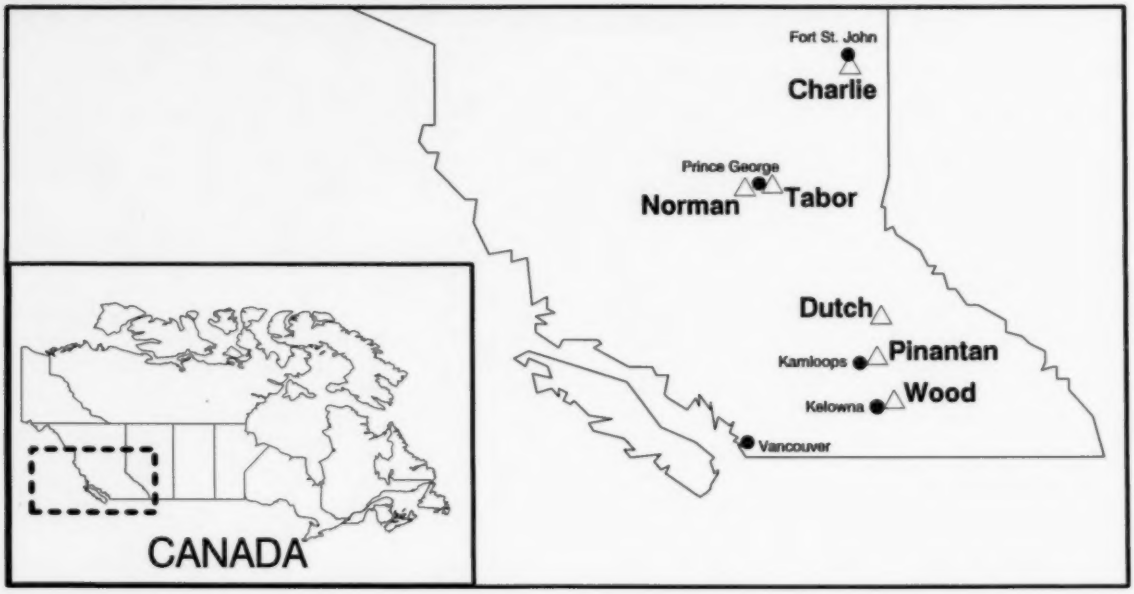
The lakes selected for this study are scattered over a wide area of inland B.C. (Fig. 1, Table 1), and most were suspected of becoming mesotrophic or eutrophic as a result of anthropogenic activities (B.C. Environment, unpublished data). Three of the lakes were of interest to B.C. Environment (Lands and Parks) because they have highly developed watersheds (Charlie), they have productive macrophytic and cyanobacterial communities (Charlie and Tabor), or they may be subject to increased land use and related impacts following the introduction of electrical power (Norman). Wood Lake, in the Okanagan basin, has been notably affected by human influence over the last few decades and has been the subject of much research (Walker et al. 1994; Stockner and Northcote 1974). Pinantan and Dutch lakes are presently eutrophic, and they were arbitrarily selected for study from a list of problem lakes (B.C. Environment, unpublished data). All of the lakes would be considered mesotrophic to eutrophic by general North American standards (Wetzel 1983). However, as explained below, they differ greatly in many respects.

Okanagan Valley

The Okanagan basin is a U-shaped valley where over 100 headwater lakes support a valuable sports fishing industry. The lowest lands, which contain the largest lakes, support considerable cottage and tourist industries as well as several dairy farms. The middle benchlands contain fertile soil with fruit orchards. The uppermost open forest lands are predominantly used for cattle grazing.

Wood Lake is somewhat unusual for this region because the history of European settlement in the watershed is well known for the past century. The catchment has been highly disrupted. Natural vegetation has been replaced by orchards and pasture land, and the southern shore is now surrounded by summer homes. The retention time of the lake may have been reduced by the installation of a pumping station in 1971 (Gray and Jasper 1982). Wood Lake receives

Fig. 1. Locations of the six study lakes (open triangles) in British Columbia, Canada. Limnological characteristics are summarized in Table 1.



nutrients primarily from leaking septic tanks and Vernon Creek, which drains agricultural lands (Walker et al. 1994). Stein and Coulthard (1971) speculated that Wood Lake was naturally eutrophic, but this has not been firmly established because long-term limnological measurements are not available. One recent study shows that specific eutrophication events in Wood Lake's catchment are closely associated with trends in the sedimentary diatom and chironomid fossil remains (Walker et al. 1994). We present the diatom data again here because quantitative [TP] reconstructions have not yet been performed for Wood Lake.

Kamloops area

Dutch Lake is located north of Clearwater in a popular tourist region. Information on the trophic status of Dutch Lake was first collected in the early 1970s when Lovell and Sackey (1973) attributed the poor flavour of fish to the excessive growth of blue-green algae. Shortly thereafter the Thompson–Nicola Regional District and the Clearwater Improvement District requested that the lake's water quality be evaluated (Nordin 1982). The filling time of the lake was estimated at 20 years; since closure of the only outflow by road construction, most of the lake's water loss is by evaporation, hence the term filling time. Because virtually no nutrient material can be lost from the lake basin, the lake is very susceptible to nutrient inputs. Hypolimnetic oxygen deficits, high nutrient concentrations, and dense algal blooms have been noted. Numerous septic tanks appear to be the most probable source of nutrients (Nordin 1982). In the last decade, considerable development has occurred north of the lake. No long-term limnological data, however, are available for this period, except for some very recent measurements.

Less information is available concerning Pinantan Lake, except that it is presently a eutrophic system (R. Nordin, B.C. Environment, Victoria, B.C., personal communication). Impacts from development and tourism are similar to those affecting Dutch Lake. However, Pinantan has a relatively small drainage basin and presumably has been less affected by anthropogenic nutrient inputs.

Prince George region

The economic activity surrounding Prince George consists largely of forest-related industries. Beef and other livestock industries are also active (Collins 1985). Two lakes near Prince George were chosen for detailed paleolimnological analyses.

Norman Lake is mesotrophic (Table 1) and receives a higher than permissible annual phosphorus load of 1213 kg/year (Carmichael 1992; Dillon and Rigler 1975). The north shore is developed with about 140 seasonal-use cabins, most with outhouses. Carmichael (1992), after assessing the general limnology of Norman Lake, recommended land-use strategies to decrease anthropogenic nutrient inputs. He also suspected, however, that much of the phosphorus load to the lake came from natural land sources and precipitation. Unfortunately, no monitoring of trophic variables (e.g., [TP], chlorophyll *a* concentration, Secchi depth) was performed before 1988.

Tabor Lake, located approximately 11 km east of Prince George along the Yellowhead Highway, has a spring [TP] (Table 1) similar to that in Norman Lake; however, Tabor Lake has higher mean summer chlorophyll *a* level and lower water clarity. Therefore, Tabor Lake is considered eutrophic (N.B. Carmichael, unpublished data). Tabor Lake has experienced severe midsummer algal blooms. Also,

the dramatic spread of the macrophyte *Elodea canadensis* over recent years is believed to be responsible for a major fish kill in 1993. This worsening condition is believed to be recent, but long-term data are not available. Nutrient inputs are suspected to originate from up to 166 domestic residences, from sawmill burners that operated until the 1960s, and from general aerial loading associated with the development of Prince George. Also, in 1961, a significant fire occurred in the lake's watershed, and in the early 1970s, the diversion of an inflow stream resulted in a longer water replacement time (N.B. Carmichael, unpublished data).

Fort St. John area

Charlie Lake is one of the most eutrophic freshwater lakes in British Columbia. It is one of seven aquatic systems in the Peace River area that has been selected for water quality assessment and development of rehabilitation objectives (Nordin and Pommen 1985), primarily because the lake provides the city of Fort St. John (population 20 000) with fisheries, recreation, industrial, and domestic water resources. There is concern that agricultural inputs and the disposal of sewage directly into groundwater from domestic residences have been providing the lake with nutrient-rich effluent. Water quality assessments of Charlie Lake have shown that trophic variables are at highly undesirable levels (Table 1), and dense blue-green algal (*Aphanizomenon flos-aquae*) blooms have occurred over at least the last two decades (N.B. Carmichael, unpublished data). Unfortunately, historical records on the lake's trophic status date back only to the mid-1970s.

Materials and methods

Coring procedures

Charlie, Norman, and Tabor lakes were cored in the late summer of 1991 by B.C. Environment personnel. Wood Lake was sampled in the summer of 1992 (Walker et al. 1994). Pinantan and Dutch lakes were cored in the spring of 1993.

The sediment cores, ranging from 27 to 45 cm in length, were collected using a modified Kajak–Brinkhurst (KB) gravity corer equipped with a 6.35 cm inside diameter core tube (Glew 1989). Sites were located in or near the deepest part of each lake. Cores were immediately sectioned on shore using a close-interval extruder (Glew 1988) and 1-cm slices were stored in Whirlpak® bags prior to subsampling. In the laboratory, subsamples of sediment were taken for ^{210}Pb dating and diatom preparation.

^{210}Pb dating

Sediment subsamples (approx. 30 g) of selected intervals were weighed, oven dried (24 h at 110°C), and ground to a fine dust. Samples were reweighed to determine dry weight and submitted to Atomic Energy of Canada Limited (Chalk River, Ont.) for ^{210}Pb analysis.

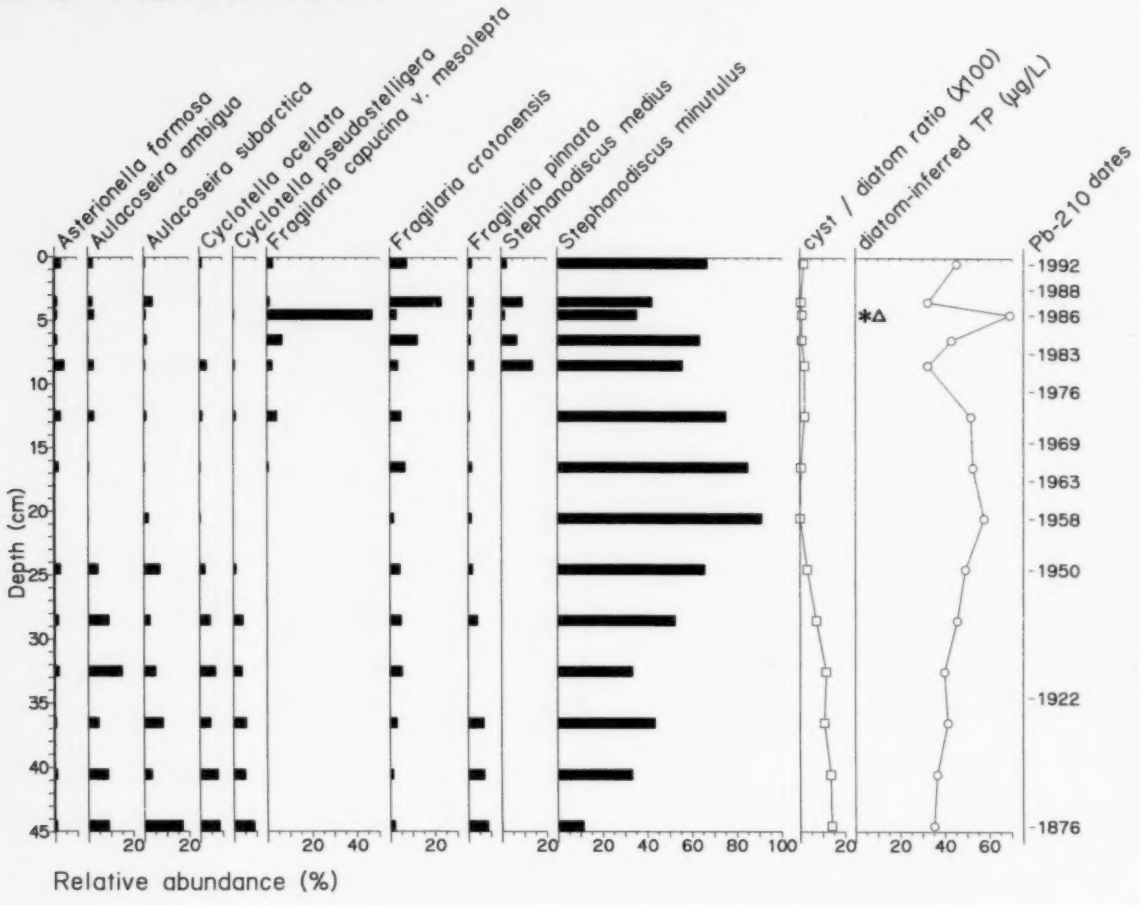
^{210}Pb dating is calculated from determinations of ^{210}Po, a decay product of ^{210}Pb. Quantitative measurements were made using alpha spectroscopy (Cornett et al. 1984). Unsupported ^{210}Pb was calculated by subtracting supported ^{210}Pb (the baseline ^{210}Pb activity naturally present in the sediments)

Table 1. Environmental characteristics of the study lakes.

		Physical					Chemical					
Lake	Location	Surface area (ha)	Max. depth (m)	Mean depth (m)	Watershed area (km^2)	Altitude (m)	Chlorophyll *a* (μg/L)	pH	Conductivity (μS/cm)	[TP] (μg/L)	Diatom-inferred [TP] (μg/L)
Wood	50°10′N, 119°38′W	930	34	21.5	190	390	4.1	8.3	325	45.4	46
Pinantan	50°75′N, 120°00′W	73	19	10	10	853	—	7.8	420	54	22
Dutch	51°65′N, 120°02′W	65.5	39	14.4	3.9	765	3.0	8.1	235	46	18
Charlie	56°40′N, 121°00′W	3120	12	5	298	691	47.3	8.1	169	55	62
Tabor	53°55′N, 122°33′W	417	9	5.4	44	702	7.6	7.8	145	25	47
Norman	53°47′N, 123°22′W	510	24	12	129.4	876	4.5	7.8	137	25	18

Note: The most important variables, according to Reavie et al. (1995), are presented. Chlorophyll *a* data were unavailable for Pinantan Lake. Values for actual [TP] are averages of available spring data, whereas values for all other variables are derived from all available data. Included are the inferred [TP] values from the uppermost sediment intervals of the study lakes.

Fig. 2. Diatom, stomatocyst/diatom ratio and inferred [TP] stratigraphies for Wood Lake. *△, the interval with poor fit to TP and a poor analogue to the calibration set.



from total activity at each level. Dates were then determined from unsupported isotopes using the constant rate of supply model (Appleby and Oldfield 1978); a computer program designed by Binford (1990) was used to perform these calculations. ^{210}Pb dating is limited to approximately 150 years before present, so extrapolations beyond this period were made on the basis of calculated sediment accumulation rates for the lowest intervals of the core. Varves were counted to determine the chronology of Pinantan Lake and charcoal particles were used to confirm the recent chronology of Tabor Lake.

Diatom preparation

Twelve evenly spaced intervals were selected from each core for diatom analysis. Extra intervals were added later for some cores, for which better stratigraphic resolution was desired. Subsamples of wet sediment (0.3–1.0 g) were heated for 1 h in a mixture of potassium dichromate and sulphuric acid to digest organic matter. Samples were then repeatedly washed in distilled water and centrifuged until they were clear and free of residual acid. The siliceous remains were settled onto cover slips, and the cover slips were mounted on glass slides using Hyrax®. For each slide, at least 500 diatom valves were identified and counted along transects under oil immersion at 1000× magnification. Diatom taxonomy was based primarily on Krammer and Lange-Bertalot (1986, 1988, 1991*a*, 1991*b*), Camburn et al. (1984–1986), and Patrick and Reimer (1966). Chrysophyte cysts were also enumerated and expressed relative to the diatom sum (Smol 1985).

Inferring total lake-water phosphorus

The transfer function we used was constructed by determining the relationship between water chemistry variables and diatom distributions in the surface sediments of 59 B.C. lakes (Reavie et al. 1995). Canonical correspondence analysis (CCA) was used to identify environmental variables with strong correlations to modern diatom assemblages (Ter Braak 1990*a*, 1990*b*). Lake-water [TP] had a significant influence on the diatom communities in our lakes, so weighted averaging calibration (Line et al. 1994) was used to estimate the [TP] optima for common diatom taxa. The

final regression equations were based on the [TP] optima of the 150 most common diatom taxa found in the 59 calibration lakes (Reavie et al. 1995).

Quantitative [TP] inferences were performed using the computer program WACALIB version 3.3 (Line et al. 1994). The distribution of [TP] measurements for the calibration set was skewed toward the oligotrophic extreme of the spectrum, so calculations were performed using transformed (ln(TP + 1)) data (see Birks et al. 1990 for details). The inferred [TP] values furnished by WACALIB were subsequently back transformed.

Fit to [TP] and modern analogue measures

A CCA constrained to [TP] was used to evaluate our [TP] reconstructions (Reavie et al. 1995). The residual distance of diatom assemblages to the TP axis provided a measure to assess lack of fit to TP. First, CCA determined what were extreme residual distances from the TP axis on the basis of modern samples with environmental data (Reavie et al. 1995). Core samples were then run passively in CCA and positioned, by means of transition formulae (Ter Braak 1990*a*), with respect to the TP axis. Fossil samples with residual distances greater than the 90 and 95% confidence limits of the calibration set were considered to have poor and very poor fits to TP, respectively.

Fossil samples with close modern analogues are more likely to provide reliable inferred [TP] values (Birks et al. 1990). Using the computer program ANALOG (J. Line and H.J.B. Birks, unpublished program), we performed analogue matching to identify fossil diatom assemblages with poor analogues to modern assemblages in the training set. Details of this method are described by Hall and Smol (1993).

Results and discussion

Okanagan Valley

The pre-20th century sediments of the Wood Lake core were dominated by a complex of planktonic diatoms, primarily *Aulacoseira ambigua*, *Aulacoseira subarctica*, *Cyclotella ocellata*, *Cyclotella pseudostelligera* (Krammer and Lange-Bertalot 1991*a*), and *Stephanodiscus minutulus* (Fig. 2). Total phosphorus inferred from this assemblage indicated that the lake already had relatively high spring nutrient levels ([TP] = 35 μg/L) before cultural impacts occurred in the drainage basin. *Stephanodiscus minutulus*, a eutrophic taxon (Håkansson 1976), increased considerably from the late 19th century until about 1958, when it constituted 93% of the diatom assemblage. Chrysophyte stomatocysts gradually decreased relative to the diatoms; cyst to diatom ratios declined from 0.14 in the older sediments (~1876) to trace amounts by about 1958. During this same period, inferred [TP] increased from 35 to 58 μg/L.

The eutrophication indicated by these trends is corroborated by other studies. In 1974, a study of the Okanagan basin suggested that Wood Lake had begun to rapidly deteriorate during the early 1900s because of agricultural development and diversion of inflow streams for irrigation (British Columbia Water Resources Service 1974). Profundal chironomids also disappeared from our sediment core around 1940 (Walker et al. 1994). Such an occurrence is characteristic of hypolimnetic anoxia resulting from eutrophication.

Diatom-inferred [TP] and the relative abundance of *Stephanodiscus minutulus* decreased after 1958, indicating that water quality improved. The opening of a Hiram Walker distillery in about 1970 is considered to be the most important event to have affected Wood Lake in recent years. Since 1971, the distillery has been pumping large volumes of water from nearby Okanagan Lake (average [TP] in the 1980s ≤21 μg/L) for cooling, and releasing it into Vernon Creek, upstream from Wood Lake (British Columbia Water Resources Service 1974). This pumping resulted in a reduction of the theoretical water replacement time from 30 to 14 years. Although this estimate varies because of precipitation (Gray and Jasper 1982), it is believed that the distillery's release of Okanagan Lake water is largely responsible for the water quality improvement (Walker et al. 1994).

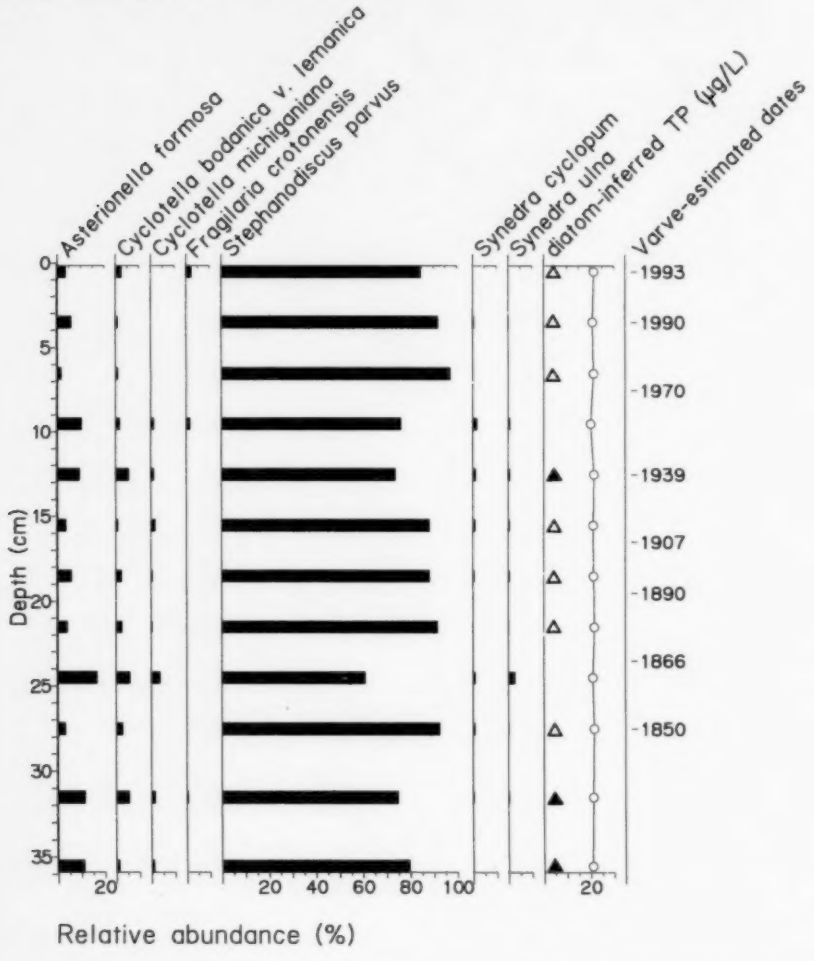
Little Round Lake, in southeastern Ontario, experienced similar changes in its recent history (Smol et al. 1983; Christie and Smol 1993). As anthropogenic nutrient enrichment occurred in Little Round Lake, the relative abundance of small *Stephanodiscus* spp. increased. Subsequently, most of the disturbance in the drainage basin ceased, and seepage from road salt enhanced meromixis, thus preventing the resuspension of hypolimnetic nutrients. Surface water quality improved and eutrophic taxa decreased in relative abundance. Water quality recoveries as dramatic as those in Wood and Little Round lakes have rarely been observed.

The lower diatom-inferred [TP] values during the past 25 years in Wood Lake were interrupted at the 4- to 5-cm interval with a sudden increase to 70 μg/L, in conjunction with a sudden increase in *Fragilaria capucina* var. *mesolepta*. The brief (2 or 3 year) bloom of *F. capucina* var. *mesolepta* may have been caused by an irregularly high nutrient load in Wood Lake at that time. However, a misleading [TP] inference may have occurred at this interval because of poor assemblage fit to [TP] (i.e., a poor analogue). This taxon is considered periphytic, so it is possible that it is responding to other changes in the ecosystem (e.g., macrophyte growth). Unfortunately, no historical information is available to explain this incident. Overall, we conclude that Wood Lake was fairly productive before the commencement of human impacts, European development caused a degradation in water quality, and recent unintentional mitigation (water pumping) has resulted in a reduction of TP concentrations.

Kamloops area

The Pinantan Lake core was varved, and on the basis of the sediment laminations, the core dates to well before 1850 (Fig. 3). *Stephanodiscus parvus*, a well-known eutrophic indicator (Anderson et al. 1990; Christie and Smol 1993), occurred in relative abundances of 60–96% throughout the core, suggesting that Pinantan is naturally eutrophic and that this has changed little since European settlement. In general, the assemblages did not shift markedly, except for a minor peak of *Asterionella formosa* during the 1850s. Similarly, the inferred [TP] values were almost uniform (at about 21 μg/L) throughout the core. However, few

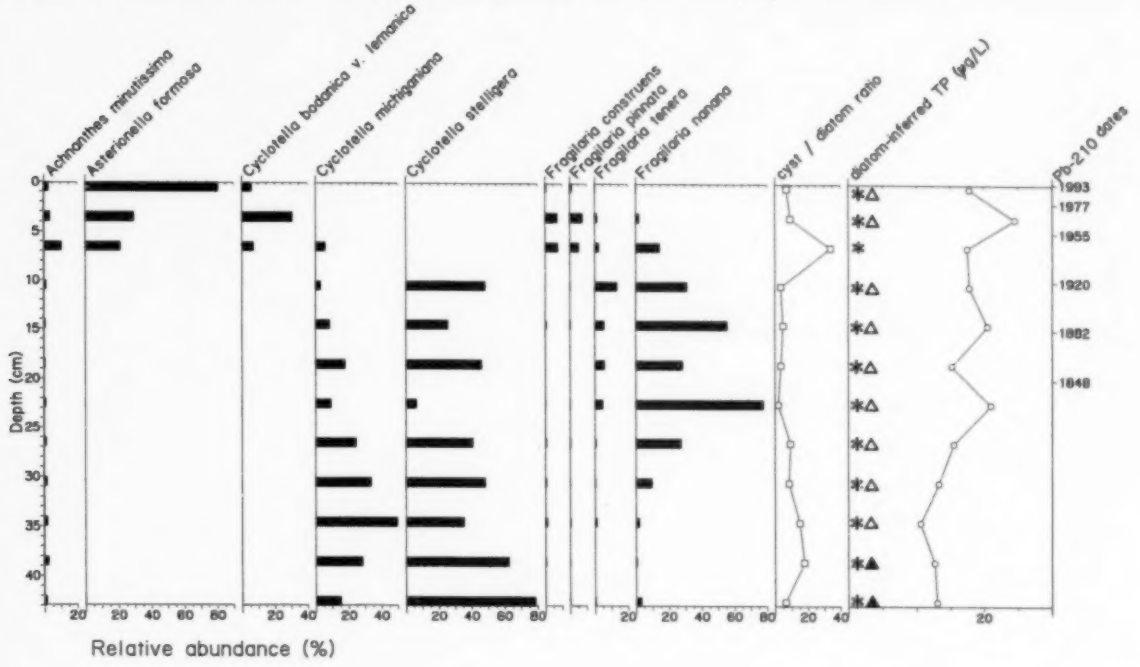
Fig. 3. Diatom and inferred [TP] stratigraphy for Pinantan Lake. Assemblages are designated on the inferred TP graph as poorly fitted to TP (▲), very poorly fitted to TP (△), and poor analogue to the calibration set (*).



analogous assemblages (with such a high relative abundance of *Stephanodiscus parvus*) occurred in our calibration set, and therefore many of the intervals showed poor fit to [TP]. Because of this, we suspect that our inference model underestimated TP concentrations. In summary, the diatom data indicate that there has been little anthropogenic impact on [TP] in Pinantan Lake.

The Dutch Lake core recorded five major diatom community shifts (Fig. 4). *Cyclotella stelligera* occurred in high relative abundance at the bottom of the core. *Cyclotella michiganiana* gradually increased in abundance until the 34-cm depth, after which it gradually tapered off to trace amounts. *Fragilaria nanana* sharply increased from 10 to 78% between the 30- and 22-cm (~1850–1920) intervals. Little is known about the ecology of *F. nanana*, but Reavie et al. (1995) described this taxon as having a relatively high [TP] optimum, indicating that eutrophication may have occurred between 1850 and 1920. From 22 to 10 cm, *F. nanana* and *C. stelligera* were dominant, but above 10 cm both species declined in the sediments. *Asterionella formosa* and *Cyclotella bodanica* var. *lemanica* increased in the uppermost 38 years of sediments, with *Asterionella formosa* dominating the 0- to 1-cm assemblage (80% relative abundance). The cyst to diatom ratio gradually decreased from 0.17 (at the 38- to 39-cm interval) to about 0.04 (between 23 and 10 cm), but at the 6- to 7-cm interval, the ratio increased sharply to 0.34, then decreased again to low levels. Dutch Lake's microfossil history closely mirrors that of other culturally affected lakes (O'Sullivan 1992; Brugam and Vallarino 1989; Brugam 1988; Stoermer et al. 1985). For example, Brugam and Vallarino (1989) documented similar diatom shifts for Meridian Lake, Washington: a presettlement *Cyclotella* community, followed by *Synedra* spp. (during deforestation), and a later increase in *Asterionella formosa*. Their observations confirmed a strong relationship between the onset of human

Fig. 4. Stratigraphies of siliceous microfossils and inferred [TP] for Dutch Lake. Assemblages are designated on the inferred TP graph as poorly fitted to TP (▲), very poorly fitted to TP (△), and poor analogue to the calibration set (*).



disturbance and an increase in *Asterionella formosa*. They also recorded a poor relationship between lake-water TP concentration and patterns in the diatom assemblages. Conversely, Stoermer et al. (1985) observed that substantial phosphorus loading to Lake Ontario resulted in community shifts similar to those in Dutch Lake (such as an increasing abundance of *Asterionella formosa*), so this matter is still disputable.

Inferred [TP] from the Dutch Lake core fluctuated with the diatom species changes, but an approximate increase from 13 to 20 µg/L was apparent. Even though a variety of assemblages are represented in the Dutch Lake core, many of the assemblages are poorly fit to our [TP] transfer function, and analogues to the calibration set were poor. Dutch Lake has probably been highly affected by human influence, but perhaps other limnological variables (e.g., dissolved organic carbon, total nitrogen) are also controlling diatom communities. Therefore, the diatom-inferred [TP] measurements may be somewhat insensitive to changes in the algal communities. However, as discussed in the previous paragraph, the species changes we observed in Dutch Lake probably indicate eutrophication.

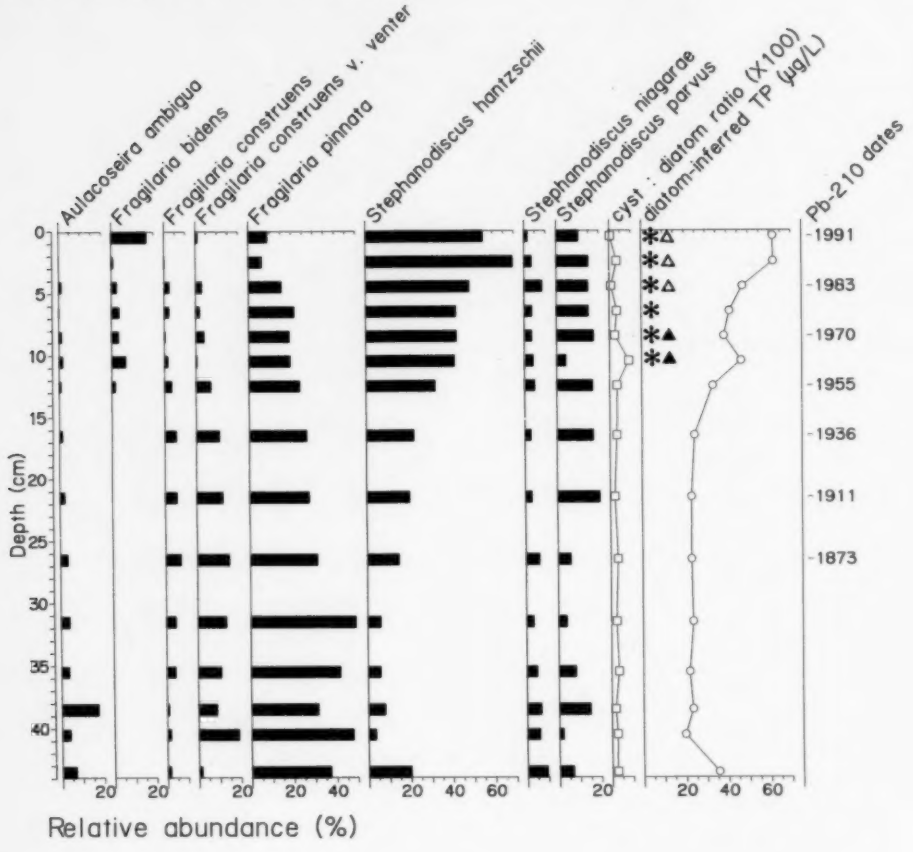
Dutch Lake is particularly interesting because it appears that increases in phosphorus loading occurred before European settlement (i.e., before 1850). By extrapolating the ^{210}Pb dates, we estimate that species shifts began ca. 1750. It is possible that the earliest recorded shift in the diatom community was due to natural causes or, more likely, that water quality changes occurred as a result of inputs from Indian settlements. Although it is known that the Shuswap tribe occupied this area well before 1800 (Balf 1969), approximate locations and populations of settlements are unknown. Therefore, the causes of this early limnological change are uncertain.

Prince George region

Diatom assemblages from the lakes in the vicinity of Prince George indicate naturally productive conditions, but the changes that have occurred since settlement differ between the two lakes (Figs. 5 and 6).

The common taxa in Norman Lake, *Aulacoseira italica* var. *subarctica*, *Fragilaria pinnata*, and *Stephanodiscus minutulus*, maintain relatively constant distributions throughout most of the core (Fig. 5). Drastic community changes have not occurred in the approximately 175 years represented by the core, and the inferred [TP] values indicate that Norman Lake is naturally mesotrophic (~24 µg/L predisturbance conditions) and has changed little since European settlement. This is not surprising because before 1970, settlement in the lake's drainage basin was limited (Carmichael 1992). *Stephanodiscus minutulus* slightly displaced other taxa after approximately 1976, suggesting a minor increase in nutrient inputs. Carmichael (1992) reported that the sewage disposal facilities (outhouses) around the lake are no more than 25 years old, so nutrient inputs from these sources are a possible cause of this slight eutrophication, as are general land disturbances associated with road and cabin construction. Our [TP] inference model appears to have been insensitive to the modest community shifts occurring within the last 15 years, and we in

Fig. 5. Stratigraphies of siliceous microfossils and inferred [TP] for Charlie Lake. Assemblages are designated on the inferred TP graph as poorly fitted to TP (▲), very poorly fitted to TP (△), and poor analogue to the calibration set (*).



fact infer slight water quality improvement. Although this contradicts our qualitative interpretation of the diatom stratigraphy, the shift is relatively minor.

In the Tabor Lake core (Fig. 6), *F. pinnata* is the most common taxon in all intervals, varying between 65% at the bottom of the core and 33% at the 1- to 2-cm interval. Many other *Fragilaria* taxa are also present, but no obvious shifts are noted. Since the mid-1970s, the eutrophic indicator *Stephanodiscus hantzschii* increased in abundance to peak in about 1985. This profile shows a trend in diatom shifts similar to that of Norman Lake, with a modest increase in small *Stephanodiscus* species to as high as 30% within the last two decades, indicating minor eutrophication. The consistent dominance of the littoral, generally ubiquitous diatom *F. pinnata* (Patrick and Reimer 1966) makes this profile difficult to interpret. *Fragilaria pinnata* is difficult to classify ecologically because it occurs in both high- and low-nutrient environments (Christie and Smol 1993; E.D. Reavie, personal observations). Tabor Lake's relative shallowness (Table 1) allows for a largely benthic assemblage to dominate.

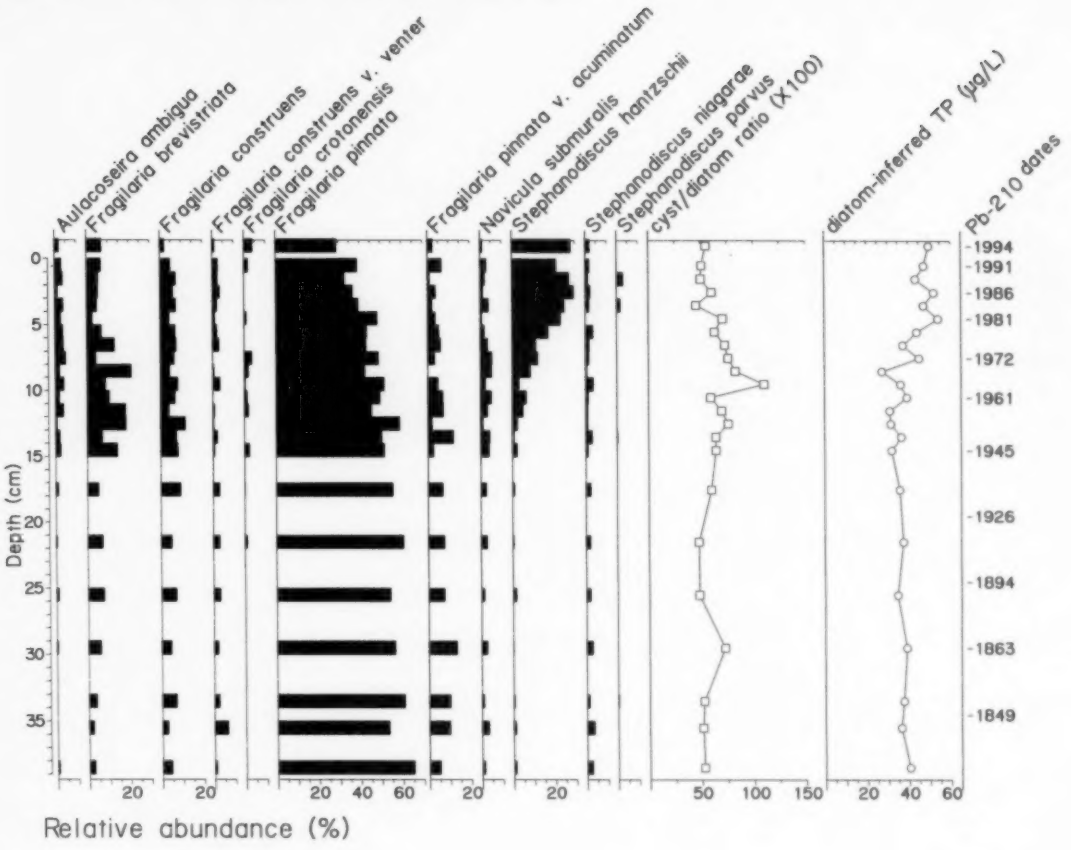
The inferred phosphorus history of Tabor Lake indicates relatively stable TP concentrations (~35 µg/L) until the 1970s, after which some modest eutrophication occurred. Another interesting trend is the apparent nutrient decrease in the uppermost two intervals, indicating that phosphorus loading may have been declining. However, a recent analysis of surface sediment (sampled in 1994; Fig. 6) shows that the relative abundance of *Stephanodiscus hantzschii* and inferred [TP] levels are similar to 1985 levels, and so water quality has probably not improved recently.

A subsequent analysis of raw sediment from the Tabor Lake core revealed numerous charcoal particles in the 11- to 9-cm depths, probably coinciding with the severe watershed fire in 1961. Nutrient inputs to the lake may have increased following the fire-induced vegetation clearance.

Fort St. John area

Aulacoseira ambigua, *F. pinnata*, and three *Stephanodiscus* species are the most common taxa in the pre-1900 sediments of Charlie Lake (Fig. 7), indicating that it was a naturally eutrophic system. The eutrophic indicator

Fig. 6. Diatom and inferred [TP] stratigraphies for Tabor Lake.



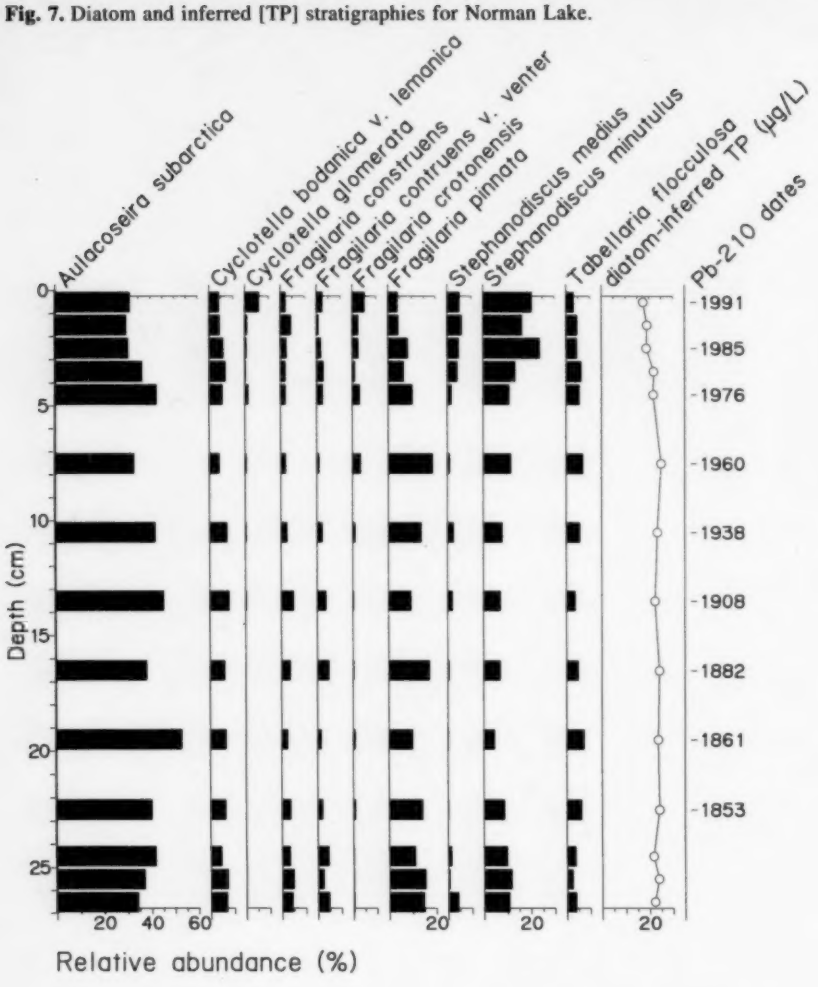
S. hantzschii increased strikingly between 1873 and 1991. *Stephanodiscus parvus* also increased slightly during this time, whereas *F. pinnata* decreased from a relative abundance of 48 to 7%. The impact of settlement was apparently severe; several papers document such dramatic increases in *Stephanodiscus* spp. with eutrophication (e.g., Osborne and Moss 1977; Anderson et al. 1990). *Fragilaria bidens* (Krammer and Lange-Bertalot 1991*a*) is not in our calibration set (Reavie et al. 1995), although it appears in the surficial sediments of Charlie Lake. Its autecology is unknown, and because it does not occur in our [TP] transfer function, our inferred [TP] was not influenced by this taxon. As expected, the most recent intervals provided poor analogues to the calibration set and contained assemblages with poor fit to [TP], probably resulting from the absence of *F. bidens* in the calibration set, and the very high relative abundances of *Stephanodiscus* spp. in the top 11 cm of the Charlie Lake core. The naturally high productivity of Charlie Lake probably prevented chrysophytes from occurring in high abundances (Sandgren 1988), as stomatocysts were found only at trace levels throughout the core.

Inferred [TP] measurements indicated an approximate 40 µg/L increase in [TP] (from 22 to 62 µg/L) since the end of the 19th century. Aquatic productivity in Charlie Lake appears to have increased during the period of deforestation, agricultural development, and sewage disposal in the region.

Evaluation of the weighted-averaging [TP] inference model

Because this is the first application of our new [TP] inference model (Reavie et al. 1995) for hindcasting long-term changes in lake-water TP concentration, it is important to assess the reliability and performance of the model. In some cases, TP concentrations inferred from surface sediment diatom assemblages closely matched available water chemistry measurements (Table 1) (Wood, Charlie, and Norman lakes), indicating that our model may be capable of providing accurate quantitative estimates of lake-water TP concentration. However, the most recent [TP] inferences from Pinantan and Dutch lakes underestimated measured values. It was inevitable that the accuracy of our [TP] inferences would be affected by the influence of other variables. The absence of analogues and lack of fit to [TP] of many fossil diatom assemblages is attributable to the effect of other factors, such as conductivity (Cumming

Fig. 7. Diatom and inferred [TP] stratigraphies for Norman Lake.



et al. 1995), which exert control over the diatom communities. This may explain the underestimated [TP] reconstructions for Pinantan and Dutch lakes. The overestimation of modern [TP] in Tabor Lake was probably caused by the high relative abundance of *F. pinnata*, a taxon with a high [TP] optimum in the calibration set. Inaccurate quantitative reconstructions are not a fatal problem, however. The addition of eutrophic lakes to the original calibration set (Hall and Smol 1992) allowed us, by providing more analogues, to more realistically infer [TP] in Wood Lake (Reavie et al. 1995). Presumably the addition of more lakes will further improve our transfer function.

General discussion and conclusions

The trends in relative abundance of siliceous microfossils provide several insights concerning the influence of cultural activities on lakes in B.C. For example, the predominant relationship between small *Stephanodiscus* taxa and high nutrient levels has been known for some time (Hustedt 1930; Håkansson 1976), and a considerable increase in *Stephanodiscus* spp. with a concomitant decline in benthic taxa has been recorded in several eutrophied lakes (Osborne and Moss 1977; Engstrom et al. 1985). Our results confirm that such a trend appears to coincide with cultural eutrophication in B.C. lakes. The diatom stratigraphy that occurred in Dutch Lake (i.e., the replacement of *Cyclotella* spp. by *Synedra* spp. and *Asterionella formosa*) is also a common indicator of anthropogenic nutrient loading (e.g., Brugam and Vallarino 1989).

The nature of some of the events in the six study lakes is not entirely clear. This may be because diatom community composition is controlled by multiple variables, making the diatom data for all of the lakes inherently "noisy." For instance, it is clear that a major episode occurred around 1986 in Wood Lake, though we have no direct knowledge of a specific historical event to explain the *F. capucina* var. *mesolepta* peak. *Fragilaria pinnata* played a major role in the [TP] inference for Tabor Lake; however, it is probable that this taxon thrives not only because it is tolerant of high [TP] (Reavie et al. 1995), but also

because its growth is optimal in shallow lakes with suitable microhabitats.

Many interesting eutrophication trends were observed in the six B.C. lakes. As expected, recent increases in phosphorus levels were inferred in some of the lakes. Others seem to have maintained their natural trophic state since pre-European settlement of inland B.C. In all cases it appears that the lakes were quite productive prior to human settlement.

The underlying hypothesis of this study was that nutrient inputs increased as human populations grew in the watersheds of every study lake. The response to this loading varied among the six lakes included in this study. The lakes with the largest catchment areas (Wood and Charlie lakes; Table 1) appear to have been the most severely affected. Hall and Smol (1993), whose studies involved a paleolimnological analysis of eutrophication following the Ontario hemlock decline (~4800 years BP), also noticed that the degree of eutrophication was related to catchment size. Apparently, forest decimation by natural causes (e.g., pathogens) has a limnological effect similar to that of human exploitation (e.g., deforestation) of the landscape.

Other variables besides catchment size obviously affected the pattern in eutrophication trends that we observed. For instance, Dutch Lake has the smallest watershed area (3.9 km^2), yet it showed more eutrophication than Norman Lake (watershed area 129.4 km^2). The relatively unaffected lakes (e.g., Norman Lake) were subjected to fewer cultural impacts than the highly perturbed systems (e.g., Dutch Lake).

Interestingly, a lake such as Pinantan that has had some development in its drainage basin (e.g., cottages, roads) showed little concomitant evidence of increased eutrophication. Similarly, Norman Lake is also naturally productive and receives nutrients mainly from natural sources (as was suggested by Carmichael (1992)). From our data, arguments could be made that possible restoration measures for these systems should be postponed until better evidence for anthropogenic eutrophication is produced. The amelioration of present human inputs to such lakes may prevent future water quality problems; however, a significant reduction in TP concentrations is probably an unrealistic goal. Conversely, Charlie Lake is an example of a severely culturally affected lake system, where remediation may improve water quality.

Acknowledgements

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Macroinvertebrate production in a headwater stream during recovery from anthropogenic disturbance and hydrologic extremes

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Abstract: Recovery of the macroinvertebrate community inhabiting a headwater stream (catchment 54; C54) that received 3 years of seasonal insecticide treatment was investigated. Estimates of abundance, biomass, and production in C54 during 1989 and 1990 were compared with those of a nearby undisturbed reference stream (catchment 55; C55), and those of C54 during a pretreatment year (1985). Total macroinvertebrate abundance was similar throughout pretreatment, treatment, and recovery periods of C54. In contrast, biomass and production, which decreased during treatment, increased to levels similar to those of C54 in the pretreatment year and those of the reference stream during recovery. By 1990, the functional structure of C54 was similar to that of C55 and that of C54 before the treatment. However, taxonomic and developmental stage differences within some functional groups, particularly shredders, persisted. Despite poor recovery of some larger shredder taxa, rapid recovery of a relatively small trichopteran shredder, *Lepidostoma* spp., contributed significantly to recovery of ecosystem processes associated with shredders. Relationships between shredder biomass and coarse particulate organic matter differed during treatment and recovery periods. Invertebrate taxa with shorter life cycles recolonized rapidly, while those with life cycles > 1 year generally displayed limited recovery. Hydrologic extremes during treatment (drought) and recovery (wet) periods affected organic matter and macroinvertebrate community dynamics in both streams, and may have influenced observed recovery patterns.

Résumé : Nous avons examiné le rétablissement de la communauté de macroinvertébrés habitant un ruisseau de montagne (captation 54; C54) qui a reçu pendant 3 ans un traitement saisonnier aux insecticides. Les estimations de l'abondance, de la biomasse et de la production dans le C54 en 1989 et 1990 ont été comparées à celles d'un ruisseau témoin non traité situé tout près (captation 55; C55) et à celles du C54 pour une année antérieure au traitement (1985). L'abondance totale des macroinvertébrés était similaire tout au long des périodes de prétraitement, de traitement et de récupération dans le C54. Par contre, la biomasse et la production, qui avaient baissé pendant le traitement, sont remontées à des niveaux similaires à ceux du C54 avant le traitement et à ceux du ruisseau témoin. La structure fonctionnelle du C54 était, en 1990, similaire à celle du C55 et à celle du C54 avant le traitement. Toutefois, des différences concernant la taxinomie et le stade de développement ont persisté dans certains groupes fonctionnels, surtout les broyeurs. Malgré le rétablissement médiocre de certains taxons broyeurs de grande taille, la récupération rapide d'un broyeur trichoptère de taille relativement petite, *Lepidostoma* spp., a joué un rôle important dans le rétablissement des processus écosystémiques associés aux broyeurs. Les relations entre la biomasse des broyeurs et les particules organiques grossières étaient différentes pendant les périodes de traitement et de rétablissement. Les taxons d'invertébrés à court cycle vital ont recolonisé rapidement, tandis que ceux dont les cycles étaient > 1 an ne manifestaient qu'un rétablissement limité. Les extrêmes hydrologiques observés pendant les périodes de traitement (sécheresse) et de rétablissement (pluie) ont affecté la dynamique de la matière organique et de la communauté d'invertébrés dans les deux ruisseaux, et ont pu influer sur les profils de rétablissement observés.
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Introduction

Studies of ecosystem recovery following disturbance provide important information on the resilience of ecosystems, information that is of great practical value for assessing and predicting effects of perturbation, as well as recovery rates and patterns (Holling 1973; Connell and Sousa 1983; Sheldon 1984; Gore et al. 1990). Additionally, these studies can have great value in elucidating relationships among biotic and abiotic components within systems, providing the basis for ecological theories such as the facilitation and inhibition models of succession (Connell and Slatyer 1977).

Although the importance of recovery studies for both practical and theoretical considerations in stream ecology is obvious, recovery patterns and assessment of recovery end points following disturbance to stream systems remain poorly understood (Kelly and Harwell 1990; Gore et al. 1990; Yount and Niemi 1990; Niemi et al. 1993). Factors contributing to this lack of understanding include differences in the recovery processes associated with different types of disturbance (Bender et al. 1984; Niemi et al. 1990, 1993) and variability in response of physically and (or) biotically different streams to similar types of disturbance (Resh et al. 1988). Additionally, there is a general lack of studies that thoroughly examine community dynamics before, during, and following disturbance, while making comparisons with similar undisturbed reference streams (see review by Yount and Niemi 1990). Thus, separating disturbance effects and recovery processes from other sources of variability (e.g., temperature and hydrologic fluctuations) is often difficult.

Recent studies have demonstrated that recovery of macroinvertebrate communities in streams is dependent upon a variety of factors including (*i*) the nature of the disturbance (length, size of area affected, duration, etc.) (Bender et al. 1984; Niemi et al. 1990); (*ii*) physical characteristics of the disturbed stream (Robinson et al. 1993); (*iii*) proximity of the disturbed stream to source areas of colonists (Cushing and Gaines 1989); and (*iv*) characteristics of the inhabitant biota including vagility and life history specifics (Wallace 1990; Mackay 1992). Additionally, recovery of some macroinvertebrate groups (e.g., filter feeders and collector gatherers) may be dependent upon recovery of other groups, particularly shredders, which influence detritus resource availability (Wallace et al. 1986, 1991*a*).

A previous study examined the effects of insecticide treatment on macroinvertebrate production in a southern Appalachian Mountain headwater stream (Lugthart and Wallace 1992). During insecticide treatment, insect abundance, biomass, and production were greatly reduced relative to pretreatment levels, while noninsect taxa proliferated in the disturbed stream. Whiles and Wallace (1992) subsequently examined invertebrate abundance and biomass in this stream during the first recovery year, and noted differences from pretreatment and reference stream communities that persisted after 1 year of recovery.

The objectives of this study were to examine macroinvertebrate community dynamics in the disturbed stream studied by Lugthart and Wallace (1992) during 2 years of recovery following insecticide treatments. Because estimates of production give insight into community structure and dynamics (Benke 1984, 1993; Lugthart and Wallace 1992), we have used production estimates, along with estimates of abundance and biomass, to examine recovery. Comparisons are made with an adjacent, undisturbed reference stream, as well as previously published production estimates from the same stream before and during insecticide disturbance (Lugthart and Wallace 1992). Abundance, biomass, and annual production estimates of the total invertebrate community, as well as functional and taxonomic groups were examined during this 2-year recovery period. Additionally, to examine relationships between invertebrate recovery and organic matter dynamics, benthic organic matter was measured throughout the study period in both the disturbed and reference streams.

Table 1. Physical characteristics of catchments 54 and 55.

	Catchment 54	Catchment 55
Catchment		
Area (ha)	5.5	7.5
Elevation (m above sea level)	841	810
Channel		
Length (m)	282	170
Gradient (cm/m)	33	20
Substrate composition (%)		
Bedrock outcrop	35.2	13.1
Boulder	3.6	2.7
Cobble	17.2	29.0
Pebble and sand	12.1	49.0
Silt	31.9	6.1
Average discharge (L/s)		
1985–1991	1.35	1.53
1989	1.97	2.34
1990	1.99	2.48
Temperature (°C)		
5-year average (1986–1990)	12.0	12.1
Annual degree-days	4402	4464

Note: Elevations were measured at gauging flumes.

Study sites

The two study streams are located at the Coweeta Hydrologic Laboratory (U.S. Forest Service), a 1625-ha drainage basin located in the Blue Ridge province of the Southern Appalachian Mountains in Macon County, North Carolina. Both streams are first order and drain catchments (C) 54 and 55. Catchments at Coweeta are typically forested with mixed hardwoods dominated by white oak (*Quercus alba*), red oak (*Quercus rubra*), red maple (*Acer rubrum*), and tulip-tree (*Liriodendron tulipifera*). Low-order streams are heavily shaded by dense riparian rhododendron (*Rhododendron maxima*) growth throughout the year. Detailed descriptions of the Coweeta basin are presented by Swank and Crossley (1988). The physical characteristics (e.g., discharge, gradient, and temperature) of the two study streams are similar and are summarized in Table 1.

Prior to this study (1986–1988), C54 was treated seasonally (four seasonal treatments per year) with the insecticide methoxychlor (1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)

ethane; Chemical Abstracts Service (CAS) No. 72-43-5). Hand sprayers were used to treat the entire length of the stream channel from the flume to the spring seep at a rate of 10 mg/L, on the basis of flume discharge. Insecticide treatment induced massive invertebrate drift (Wallace et al. 1989, 1991*b*). Abundance, biomass, and production of insects during the first year of treatment were reduced relative to pretreatment values by 72, 75, and 80%, respectively (Lugthart and Wallace 1992), and large accumulations of unprocessed leaf litter were present in C54 by the final treatment year (Wallace et al. 1995). In contrast to insects, noninsect abundances and production increased by 35 and 40%, respectively, during treatment. However, because of the loss of decapods, noninsect biomass decreased during treatment by approximately 40%.

Seasonal treatment of C54 continued for 3 consecutive years (1986–1988), with the final treatment occurring in October 1988. Recovery sampling commenced in December 1988, corresponding with what would have been the final winter insecticide application.

During the 1985–1988 treatment period of C54, record drought conditions prevailed, with 1986 having the lowest annual precipitation (124 cm) recorded at Coweeta (69% of the 60-year average of 180.1 cm) (Coweeta Hydrologic Laboratory, unpublished data). In contrast, the 1989 recovery sampling year was the wettest on record (234 cm), and was followed by another extremely wet year (1990; 209 cm). Although precipitation during 1990 was slightly lower than that of 1989, discharge in both study streams was higher during 1990 (Table 1). Undoubtedly, a large portion of 1989 precipitation was apportioned to groundwater recharge following the preceding drought period.

Methods

Benthic sampling

Benthic samples were collected monthly (C54) and bimonthly (C55) for a 2-year period commencing in December 1988. Four mixed substrate and three bedrock outcrop samples were collected on each sampling date. Prior to each sampling date, sampling sites within each stream were randomly selected.

Mixed substrate habitats (composed of various mixtures of sand, gravel, pebble, and cobble) were sampled with a 400-cm^2 stovepipe-style coring device. The corer was driven ca. 10 cm (or until bedrock was contacted) into areas of mixed substrates and all material was removed by hand and cup. Bedrock outcrop habitats were sampled by scraping and brushing all associated material from a 15 × 15 cm area into a large plastic bag held against the rock surface at the downstream margin of the sampled area.

Organic components of each sample were elutriated from inorganic substrates and passed through 1-mm and 250-μm nested sieves. Samples were preserved in a 6–8% Formalin solution containing Phloxine B dye. Invertebrates in the >1-mm fraction were removed by hand sorting under a dissecting microscope using 15× magnification. Occasionally, the fraction of a sample between 250 μm and 1 mm was subsampled (1/4 to 1/64 of sample), using a sample splitter (Waters 1969), prior to removal of invertebrates under a dissecting microscope.

Invertebrates from samples were identified, counted, and measured (total body length) under a dissecting microscope equipped with a graduated stage. Insects were identified to genus and species whenever possible, except for chironomids, which were identified as Tanypodinae or non-Tanypodinae. Most noninsect taxa were identified only to order.

Following invertebrate removal, samples were processed to obtain ash-free dry mass (AFDM) estimates of benthic organic matter. Organic materials from the >1-mm fraction of each sample (coarse particulate organic material (CPOM)) were divided into leaves, wood, seeds and buds, roots, and miscellaneous categories. Materials in each category were then oven dried (60°C for approximately 7 d), weighed, ashed (500°C for 2–12 h), and reweighed to obtain AFDM estimates. Materials <1 mm (fine particulate organic matter (FPOM)) were sampled by withdrawing a 5- to 100-mL subsample from a known volume of stirred material (1–10 L), and filtering through a preashed and preweighed glass fiber filter. Filters were then dried, weighed, and ashed as described for CPOM. Total FPOM estimates included materials retained on the 250-μm sieve, materials that passed through the 250-μm sieve, and a sample of water left in the corer after substrates were removed in the field. All were processed in the same manner (filtering) and summed to obtain estimates of FPOM for each benthic sample.

Organic matter data from individual samples were used to examine differences amongst years within each stream. Because organic matter data were not normally distributed, annual CPOM and FPOM standing stocks within streams were compared by a Kruskall–Wallis analysis of variance (ANOVA) on ranks and Dunn's multiple comparison procedure ($p < 0.05$).

Biomass and production estimates

Biomass estimates (AFDM) for all insect taxa and large noninsect taxa were obtained with length–weight regressions derived from animals in the study streams, other streams at the Coweeta Hydrologic Laboratory (Huryn 1986), or other North Carolina streams (Smock 1980). Estimates made using regressions from Smock (1980) were multiplied by 0.85 to convert from dry mass to AFDM. Biomass estimates for small noninsect taxa (e.g., Copepoda, Hydracarina) were obtained by determining mean individual biomass from masses of >50 individuals in each size-class.

Annual production was estimated for all taxa collected over the study period. For most insect taxa except non-tanypodine Chironomidae, the size–frequency method was employed (Hamilton 1969). Values were corrected for the cohort production interval (CPI) according to Benke (1979). Non-tanypodine Chironomidae production estimates were obtained via the community-level method (Huryn 1990). For noninsect taxa and rare insect taxa, production was estimated by multiplying mean annual standing stock biomass by production to biomass ratios (P/B) obtained from other studies. These P/B values were 0.58 for crayfish (*Cambarus bartonii*) (Huryn and Wallace 1987*a*), 18 for Copepoda (O'Doherty 1988), 5 for other noninsect taxa (Oligochaeta, Nematoda, Hydracarina, and Turbellaria) (Waters 1977), and 5 or 10 for rare insect taxa that are univoltine or bivoltine, respectively (Waters 1977).

Table 2. Annual production (g AFDM·m^{-2}·year^{-1}) of invertebrate functional groups in mixed substrates and bedrock outcrop habitats of catchments 54 and 55 during the first (1989) and second (1990) years of recovery in C54.

	Catchment 54				Catchment 55			
	Mixed substrates		Bedrock outcrop		Mixed substrates		Bedrock outcrop	
Group	1989	1990	1989	1990	1989	1990	1989	1990
Scrapers	0.24 (2)	0.21 (1)	0.18 (4)	0.58 (13)	0.13 (1)	0.23 (3)	0.13 (2)	0.26 (5)
Shredders	2.52 (22)	3.58 (26)	1.03 (22)	1.22 (27)	3.09 (34)	2.06 (30)	1.38 (19)	2.04 (36)
Gatherers	5.44 (47)	3.82 (27)	1.67 (35)	1.70 (37)	2.30 (26)	2.05 (30)	1.91 (26)	1.20 (21)
Filterers	0.51 (4)	0.73 (5)	1.15 (24)	0.58 (13)	0.29 (3)	0.71 (10)	2.80 (39)	1.72 (31)
Predators	2.94 (25)	5.69 (41)	0.70 (15)	0.48 (10)	3.21 (36)	1.83 (27)	1.00 (14)	0.37 (7)
Total	11.65	14.03	4.73	4.56	9.02	6.88	7.22	5.59

Note: Values in parentheses are the percent contribution of each group to total production.

All taxa were assigned to a functional feeding group on the basis of Merritt and Cummins (1984) or our knowledge of local fauna. Crayfish abundance, biomass, and production were divided among shredders (one half), collector–gatherers (one quarter), and predators (one quarter) according to Huryn and Wallace (1987*a*).

Mean annual abundance, biomass, and production for each taxon, functional groups, and the total invertebrate community were estimated separately for the two major habitats within each stream (mixed substrates and bedrock outcrop). Values were then habitat weighted for the proportion of each habitat in each stream. When comparisons were made between streams, one-way ANOVA ($p < 0.05$) was used. Prior to analysis with ANOVA, data were $\log(X + 1)$ transformed to eliminate heteroscedasticity (Zar 1984). Flexible strategy cluster analysis ($\alpha = -0.25$) (Ludwig and Reynolds 1988) was used to examine similarities between invertebrate communities (total invertebrates and functional groups) in C54 during pretreatment, treatment, and recovery periods. Relationships between invertebrate biomass and benthic organic matter standing stocks were investigated with simple linear regressions performed on $\log(X + 1)$ transformed data using individual samples from each year.

Results

Habitat comparisons

Invertebrate production in mixed substrate habitats exceeded that of bedrock outcrops during 1989 and 1990 by more than 2 times in C54 and to a lesser degree in C55 (Table 2). Shredders, collector–gatherers, and predators codominated mixed substrate production in C55 during both 1989 and 1990 (Table 2). However, collector–gatherers dominated production in C54 mixed substrates in 1989, exhibiting production more than 2 times greater than that of collector–gatherers in mixed substrates of C55 during 1989. Collector–gatherer production decreased in C54 mixed substrates during the second recovery year, while that of shredders and particularly predators increased. These increases in C54 coincided with decreases in shredders, predators, and collector–gatherers in mixed substrates of C55 (Table 2). As a result, invertebrate production in C54 mixed substrates was more than 2 times higher than in C55 during 1990.

Invertebrate production on bedrock outcrop habitats in the C55 reference stream was dominated by collector–filterers during 1989 and by shredders and collector–filterers during 1990 (Table 2). However, as observed for mixed substrate habitats in C54, collector–gatherers displayed the highest production on bedrock outcrop habitats in C54 during 1989, followed by collector–filterers. Collector–gatherers continued to dominate bedrock outcrop production in C54 during 1990, as shredder and collector–filterer production remained relatively low in comparison with that in the reference stream (Table 2). As a result, C54 bedrock outcrop production remained ca. 1 g lower than that of C55 during 1990.

Habitat-weighted invertebrate communities

Total habitat-weighted invertebrate abundance in C54 during the first recovery year (74 992 individuals/m^2) was similar to that observed during pretreatment and treatment years, and declined slightly to 56 162 individuals/m^2 by the second recovery year (Fig. 1). Total invertebrate abundances were comparable throughout pretreatment, treatment, and recovery years in C54. However, total invertebrate abundances in C54 during the 1989 and 1990 recovery years were somewhat higher than values in C55 (1.6 times higher in 1990) over the same time period (Fig. 1).

Total habitat-weighted invertebrate biomass and production estimates during the recovery period showed a much clearer trend than abundance estimates. Both biomass and production of total invertebrates in C54 increased from the treatment year to the first year of recovery by ca. 40 and 54%, respectively. These values further increased in 1990, and were similar to those observed in the C55 reference stream and C54 pretreatment (Fig. 1). However, the trend observed in C54 is opposite to that observed in C55, where total invertebrate biomass and production values decreased during the record wet years of 1989 and 1990 (Fig. 1).

The contribution of insects to total invertebrate abundance, biomass, and production in C54 increased during recovery, as noninsect values dropped and insect taxa recovered (Fig. 1). The percent contribution of insects to

Fig. 1. Habitat-weighted abundance (individuals/m^2), biomass (g AFDM/m^2), and production (g AFDM·m^{-2}·$year^{-1}$) of insect and noninsect invertebrates in treated (catchment 54) and reference (catchment 55) streams during 1985 (pretreatment), 1986 (treatment), 1989 (first recovery year), and 1990 (second recovery year). Data for 1985 and 1986 are from Lugthart and Wallace (1992).

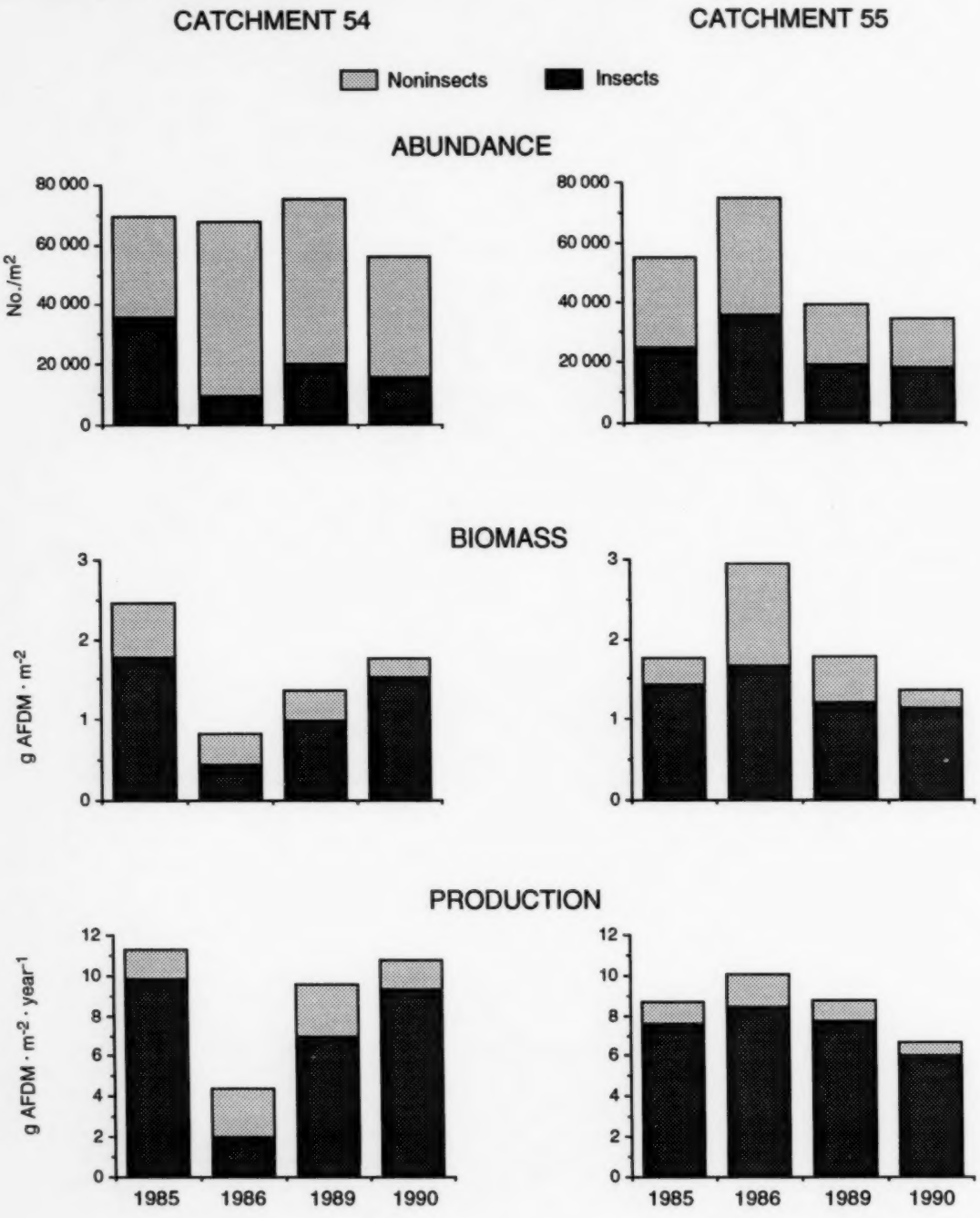
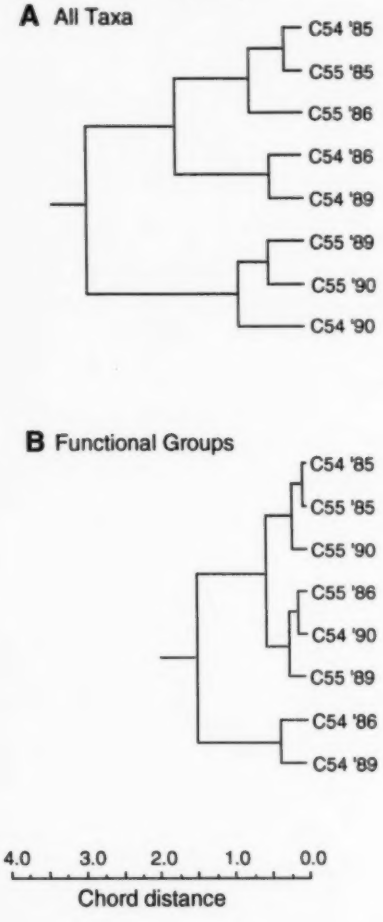


Fig. 2. Dendrograms generated from flexible strategy cluster analysis ($\alpha = -0.25$) performed on habitat-weighted annual production of all invertebrate taxa (A) and functional groups (B) in catchments 54 and 55 (C54 and C55) before (1985) and during (1986) treatment of C54, and during first (1989) and second (1990) years of recovery in C54. Data for 1985 and 1986 are from Lugthart and Wallace (1992).



total abundance in 1990 (28%) was still somewhat lower than that in C54 during the pretreatment year (53%) and in C55 (ca. 50% for all years). However, the percent contributions of insects to biomass (87%) and production (87%) in C54 during 1990 were both similar to those observed in C54 during the pretreatment year and in C55 (Fig. 1). High noninsect biomass values observed in C54 during 1985 and C55 during 1986 were a result of the presence of a few large decapods collected in benthic samples, which have slow growth rates and are insufficiently abundant to greatly affect production and abundance values.

Cluster analysis performed on habitat-weighted production of all invertebrate taxa reflected close similarity between C54 and C55 before treatment (1985) (Fig. 2A).

Table 3. Functional composition of catchments 54 and 55 during the first (1989) and second (1990) years of recovery in catchment 54.

	Catchment 54		Catchment 55	
Functional group	1989	1990	1989	1990
Scraper				
Production	0.22 (2)	0.34 (3)	0.13 (1)	0.23 (3)
P/B	14.7	5.3	5.3	6.2
Shredder				
Production	2.00 (22)	2.77 (26)	2.87 (33)	2.06 (31)
P/B	8.0	7.4	3.9	3.6
Gatherer				
Production	4.11 (45)	3.04 (28)	2.25 (26)	1.94 (29)
P/B	8.2	8.5	7.0	8.6
Filterer				
Production	0.73 (8)	0.68 (6)	0.62 (7)	0.84 (13)
P/B	8.4	7.8	9.4	7.3
Predator				
Production	2.15 (23)	3.88 (36)	2.92 (33)	1.64 (24)
P/B	4.2	4.3	4.7	4.0
Total				
Production	9.21	10.71	8.79	6.71
P/B	6.8	6.1	5.0	4.9

Note: Values are annual habitat-weighted production ($g\ AFDM \cdot m^{-2} \cdot year^{-1}$) with percent contribution of each group to total production for that year in parentheses, and production to biomass (P/B) ratios.

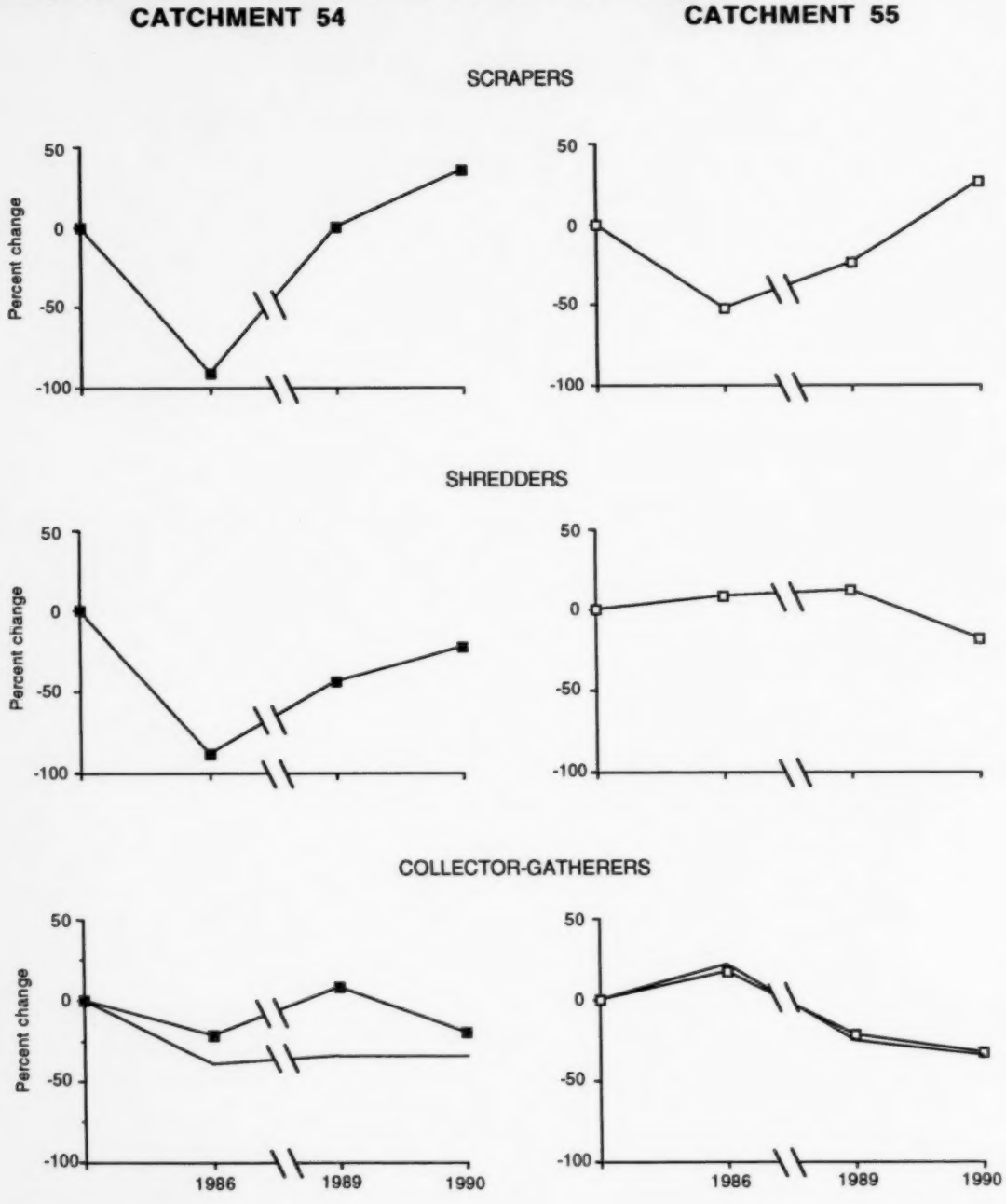
Values for the treatment (1986) and the first recovery year (1989) in C54 were also quite similar (Fig. 2A). These 2 years in C54 (1986 and 1989) then clustered with all other drought-period years, including the 1985 pretreatment year. However, values for C54 in 1990 were more similar to the results obtained during other wet years (C55 in 1989 and 1990) (Fig. 2A).

Habitat-weighted functional and taxonomic comparisons

Cluster analysis performed on habitat-weighted functional group production showed less overall difference amongst streams and years than that of individual taxa (cf. Figs. 2A and 2B). First-year recovery in C54 again grouped with the treatment year. However, these 2 years appeared to be distinctly different from all other years, and no pattern of dry and wet years was evident from functional analysis (Fig. 2B).

Production of most functional groups in C54 decreased substantially during treatment of C54, while functional groups in the reference stream showed little change during this period (Fig. 3). Collector–gatherers, which include numerous noninsect taxa (e.g., Copepoda and Cladocera), were the only group to show little treatment effect in C54. However, production of only insect collector–gatherers in C54 did decline during treatment and remained somewhat lower than pretreatment values through 1990 (Fig. 3). By 1990, only shredder production remained substantially lower than pretreatment values (22% lower), while production of other groups was similar to (filterers), or somewhat higher than, pretreatment values (Fig. 3). Predator

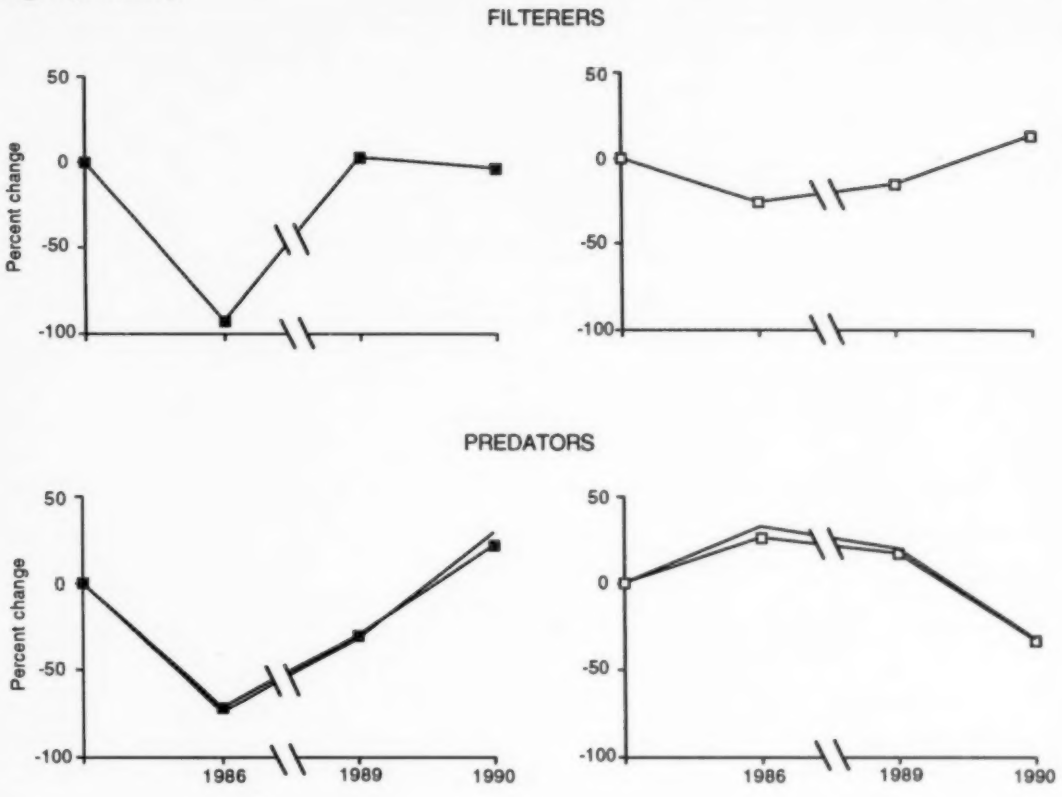
Fig. 3. Percent change of functional group production in catchments 54 and 55 during 1986 (catchment 54 treatment), 1989 (catchment 54 first-year recovery), and 1990 (catchment 54 second-year recovery) relative to 1985 (catchment 54 pretreatment year). Lines without plot symbols (collector–gatherers and predators) represent percent change in production of insect taxa only. Data for 1985 and 1986 are from Lugthart and Wallace (1992).



production increased markedly from 1989 to 1990, rising from being 30% lower than C54 pretreatment values during 1989 to being 21% higher than C54 pretreatment values during 1990.

During the first recovery year, total invertebrate production in C54 had already surpassed that of C55. However, differences in functional group production between C54 and C55 were evident. Shredder and predator production in

Fig. 3 (*concluded*).



C54 during 1989 were 70 and 74%, respectively, of that observed in C55 (Table 3). In contrast, scraper, collector–filterer, and particularly collector–gatherer production in C54 had already exceeded that of C55 in 1989, more than making up the deficit of the other three groups. By 1990, production of most functional groups in C54 was similar to, or exceeded, production in C55, while that of predators was substantially higher (2.4 times) than in C55 (Table 3).

P/B ratios reflected recovery patterns and taxonomic changes in some functional groups during recovery. During 1989, the P/B ratios of all functional groups except predators and filterers in C54 were at least slightly higher than in C55 (Table 3). Scraper and shredder P/B ratios were more than 2 times higher than those of C55, indicating the relative importance of smaller, faster growing taxa. As a result, the total community P/B ratio of C54 during 1989 was 1.4 times that of C55. By 1990, all functional group P/B ratios were similar among streams, except for shredders in C54, which remained ca. 2 times higher than those in C55 (Table 3).

During 1989, the single dominant taxon of all functional groups except filterers in C54 did not match that of C55, although the top three taxa of each group were usually similar between the two streams (Table 4). However, in the case of shredders, *Lepidostoma* spp. (Trichoptera: Lepidostomatidae) dominated production in C54 during 1989, but was not a dominant taxon (i.e., among the top three on the basis of production) in C55 (Table 4). Conversely, *Tallaperla* spp. (Plecoptera: Peltoperlidae) was not a dominant shredder in C54 during 1989 but was important in C55 during both 1989 and 1990. By 1990, the dominant taxa of all functional groups were similar between streams, with the exception of the importance of the scraping caddisfly *Neophylax mitchelli* Carpenter (Trichoptera: Limnephilidae), and the continued importance of *Lepidostoma* spp. in C54 (Table 4).

Univoltine invertebrate taxa dominated production in both streams during 1989 and 1990 (Table 5). However, trends in the two streams differed. Production of polyvoltine taxa accounted for 24% of total invertebrate production in C54 during 1989, and decreased to 14% during 1990. Production of all other groups, particularly those with life cycles >1 year, increased from 1989 to 1990 in C54 (Table 5). In contrast, production of merovoltine and univoltine taxa decreased from 1989 to 1990 in C55, and changes in polyvoltine and semivoltine groups were more subtle than those in C54 (Table 5). Thus, a trend of increasing production of taxa with relatively long life cycles was taking place during recovery of C54, while overall production was decreasing in C55.

Production of most insect orders in C54 increased from 1989 to 1990, whereas all noninsect groups declined (Table 6). Copepod abundances in C54, which were significantly

Table 4. Annual habitat-weighted production (g AFDM·m^{-2}·year^{-1}) of the three dominant taxa in each functional group in catchments 54 and 55 during the first (1989) and second (1990) years of recovery in catchment 54.

Catchment 54				Catchment 55			
1989		1990		1989		1990	
Taxon	Production	Taxon	Production	Taxon	Production	Taxon	Production
Scrapers							
Baetis	0.13 (61)	*Stenonema*	0.11 (33)	*Stenonema*	0.10 (75)	*Stenonema*	0.08 (35)
Epeorus	0.03 (12)	*Neophylax*	0.09 (26)	*Optioservus*	0.01 (09)	*Ectopria*	0.05 (21)
Ectopria	0.02 (11)	*Baetis*	0.07 (20)	*Ectopria*	<0.01 (04)	*Baetis*	0.05 (21)
Shredders							
Lepidostoma	0.83 (41)	*Tipula*	1.28 (47)	*Tipula*	1.09 (38)	*Tipula*	0.92 (45)
Tipula	0.73 (36)	*Lepidostoma*	0.93 (34)	*Pycnopsyche*	0.76 (27)	*Tallaperla*	0.45 (22)
Pycnopsyche	0.22 (11)	*Tallaperla*	0.25 (09)	*Tallaperla*	0.31 (11)	*Pycnopsyche*	0.24 (12)
Gatherers							
Oligochaeta	1.66 (40)	Chironomidae	1.02 (33)	Chironomidae	1.20 (53)	Chironomidae	1.05 (54)
Chironomidae	1.43 (35)	Oligochaeta	0.94 (31)	Oligochaeta	0.57 (25)	Oligochaeta	0.45 (23)
Copepoda	0.65 (16)	Copepoda	0.44 (14)	Copepoda	0.18 (08)	*Amphinemura*	0.15 (08)
Filterers							
Diplectrona	0.41 (56)	*Diplectrona*	0.49 (73)	*Diplectrona*	0.27 (43)	*Diplectrona*	0.54 (64)
Parapsyche	0.20 (27)	*Wormaldia*	0.10 (14)	*Parapsyche*	0.19 (31)	*Parapsyche*	0.18 (21)
Wormaldia	0.09 (12)	*Parapsyche*	0.08 (11)	*Wormaldia*	0.09 (14)	*Wormaldia*	0.12 (14)
Predators							
Lanthus	0.61 (27)	Ceratopogonidae	1.02 (27)	Ceratopogonidae	1.17 (40)	Ceratopogonidae	0.39 (24)
Dolichopodidae	0.29 (13)	*Lanthus*	0.93 (24)	Tanypodinae	0.36 (12)	nr. *Pedicia*	0.39 (24)
Ceratopogonidae	0.26 (12)	*Pedicia*	0.59 (15)	nr. *Pedicia*	0.27 (09)	*Lanthus*	0.16 (10)

Note: Values in parentheses are percent contribution to total functional group production.

Table 5. Habitat-weighted annual production (g AFDM·m^{-2}·year^{-1}) of invertebrate taxa grouped by life history in catchments 54 and 55 during the first (1989) and second (1990) years of recovery in catchment 54, and percent change from 1989 to 1990 in each stream.

	Catchment 54			Catchment 55		
	Production			Production		
Life history	1989	1990	% change	1989	1990	% change
Polyvoltine[a]	2.21	1.52	−31	1.40	1.24	−12
Bivoltine[b]	0.06	0.07	14	0.09	0.11	19
Univoltine[c]	6.04	7.45	19	6.32	4.52	−28
Semivoltine[d]	0.70	1.19	41	0.67	0.69	3
Merovoltine[e]	0.21	0.46	55	0.34	0.15	−55

[a]Examples are Copepoda, collector–gatherer Chironomidae (Diptera).
[b]Examples are Simuliidae (Diptera), *Wormaldia moesta* (Trichoptera).
[c]Examples are *Lepidostoma* spp. (Trichoptera), *Leuctra* spp. (Plecoptera), *Tipula* spp. (Diptera).
[d]Examples are *Tallaperla* spp. (Plecoptera), *Lanthus vernalis* (Odonata).
[e]Examples are Decapoda, *Cordulegaster* spp. (Odonata).

Table 6. Taxonomic composition of catchments 54 and 55 during the first (1989) and second (1990) years of recovery in catchment 54.

	Catchment 54		Catchment 55	
	1989	1990	1989	1990
Insects				
Odonata	0.82 (9)	1.39 (13)	0.26 (3)	0.25 (4)
Ephemeroptera	0.34 (4)	0.43 (4)	0.23 (3)	0.21 (3)
Plecoptera	0.30 (3)	0.82 (8)	0.68 (8)	0.73 (11)
Trichoptera	1.78 (19)	1.82 (17)	1.75 (20)	1.27 (19)
Coleoptera	0.04 (<1)	0.02 (<1)	0.03 (<1)	0.09 (1)
Diptera	3.40 (37)	4.74 (44)	4.78 (54)	3.46 (52)
Total insects	6.68 (73)	9.22 (86)	7.73 (88)	6.01 (90)
Noninsects				
Oligochaeta	1.66 (18)	0.94 (9)	0.57 (6)	0.46 (7)
Nematoda	0.03 (<1)	0.02 (<1)	0.02 (<1)	0.01 (<1)
Turbellaria	0.10 (1)	0.03 (<1)	0.03 (<1)	0.01 (<1)
Cladocera	0.03 (<1)	0.01 (<1)	0 (0)	0 (0)
Copepoda	0.64 (7)	0.44 (4)	0.18 (2)	0.14 (2)
Acari	0.07 (1)	0.05 (<1)	0.02 (<1)	0.02 (<1)
Decapoda	0 (0)	0 (0)	0.24 (3)	0.06 (1)
Total noninsects	2.53 (27)	1.49 (14)	1.06 (12)	0.69 (10)
Total invertebrates	9.21	10.71	8.79	6.71

Note: Values are annual habitat-weighted production (g AFDM·m^{-2}·year^{-1}) with percent contribution of each group to total production for that year in parentheses.

higher than those in C55 during 1989 ($p < 0.05$), decreased to levels similar to those in C55 during 1990 (Fig. 4). A similar trend was evident for abundance, biomass, and production of all noninsect taxa in C54 during recovery (see Appendix). By 1990, production of some insect orders in C54 had substantially surpassed those in C55. These included Trichoptera, Diptera, and Odonata, with the latter two more than 1 g AFDM·m^{-2}·year^{-1} higher than those in C55 (Table 6).

By 1990, production of most individual insect taxa in C54 was similar to or exceeded that of insect taxa in C55 (see Appendix). However, some important exceptions, particularly amongst shredder taxa and taxa with longer life cycles, were evident. For example, production of *Tallaperla* spp., an important leaf-shredding stonefly in undisturbed Coweeta streams, was substantially lower in C54 than C55 during 1989 and remained at only 55% of the production in C55 during 1990 (Table 4). *Tallaperla* spp. are semivoltine in Coweeta streams, with two cohorts normally present in undisturbed streams. However, size–frequency plots of *Tallaperla* spp. demonstrate that only one cohort was present in C54 for much of the 2-year recovery period (Fig. 5). In contrast, the relatively small univoltine shredding caddisflies *Lepidostoma lydia* and *Lepidostoma griseum* recolonized C54 rapidly and continued to increase throughout the recovery period. Thus, during 1990 the abundance of *Lepidostoma* spp. (both species combined) in C54 was significantly higher than that in C55 ($p < 0.05$) (Fig. 6).

Benthic organic matter

Average annual standing stocks of benthic leaf litter and total CPOM remained similar in the mixed substrate habitat of C54 during pretreatment, first-year treatment, and the first recovery year, but declined significantly during the second year of recovery ($p < 0.05$) (Table 7). This same pattern was evident in C55, suggesting that washout of CPOM from high discharge during 1990, rather than a strong recovery of leaf-shredding invertebrates in C54, resulted in lower CPOM standing stocks during 1990 (Table 7). FPOM showed a pattern similar to that of CPOM, with 1990 standing stocks of FPOM in mixed substrate habitats of both streams significantly lower than those of 1986 (first year of treatment) and 1989 (first year of recovery) ($p < 0.05$) (Table 7).

Because of generally lower values and higher variability amongst samples, changes in CPOM standing stocks on bedrock outcrop habitats of both streams were unclear (Table 7). However, bedrock outcrop FPOM values in C54 during 1989 were significantly higher than in other years ($p < 0.05$). A similar, but less significant, pattern was observed in C55 (Table 7). Because both streams are dominated by mixed substrates, habitat-weighted values of all organic matter categories showed the same pattern observed for mixed substrate habitats (lowest values during 1990) (Table 7).

Invertebrate and organic matter relationships

Collector–gatherer biomass (both total and noninsects) in both streams was positively correlated with FPOM throughout pretreatment, treatment, and recovery periods of C54 ($p < 0.05$). However, shredder biomass and CPOM relationships in C54 showed strong treatment and recovery effects (Table 8). During the 1985 pretreatment year, shredders in C54 and C55 were positively correlated with CPOM

Fig. 4. Habitat-weighted average abundance (±SE) of Copepoda in the treated (catchment 54; C54) and reference (catchment 55; C55) streams during the 2-year recovery period of C54. Asterisk indicates significant difference between pooled annual abundances (log-transformed monthly values) in each stream (ANOVA, $p < 0.05$).

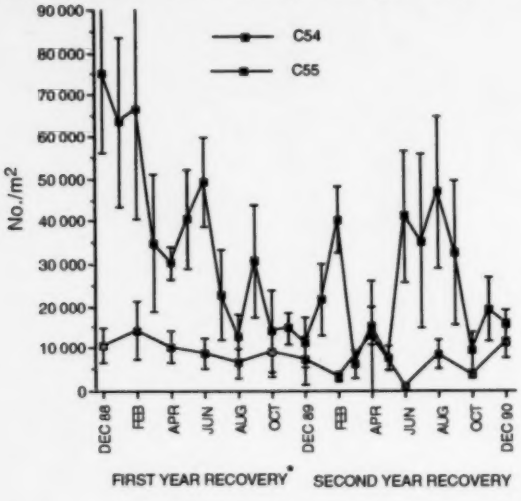
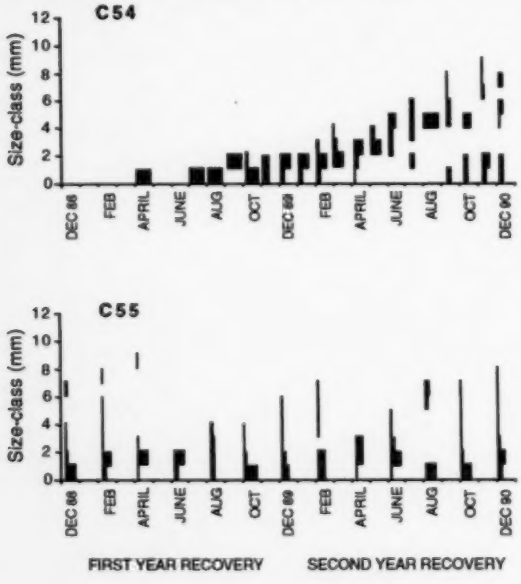
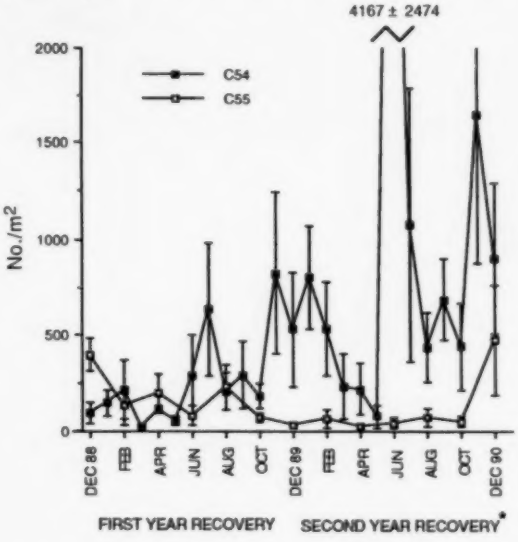


Fig. 5. Size–frequency plots, based on total body length, of *Tallaperla* sp. (Plecoptera: Peltoperlidae) in the treated (catchment 54; C54) and reference (catchment 55; C55) streams during the 2-year recovery period of C54.



($p < 0.001$). However, no significant relationship was evident between shredders and CPOM in C54 during treatment ($p > 0.05$). During 1989, a positive correlation was again evident in C54, and this relationship strengthened (higher r)

Fig. 6. Habitat-weighted average abundance (±SE) of *Lepidostoma* spp. in the treated (catchment 54; C54) and reference (catchment 55; C55) streams during the 2-year recovery period of C54. Asterisk indicates significant difference between pooled annual abundances (log-transformed monthly values) in each stream (ANOVA, $p < 0.05$).



during the second recovery year ($p < 0.001$ for both years) (Table 8). In contrast, shredder biomass and CPOM were significantly correlated in the reference stream throughout treatment and recovery years of C54 ($p < 0.001$).

Discussion

As noted by Lugthart and Wallace (1992) during treatment of C54, and Whiles and Wallace (1992) during first-year recovery, invertebrate abundance data failed to accurately depict changes occurring in C54 throughout the 1989–1990 recovery period (see Fig. 1). Abundance data alone, especially when considered as a whole (total invertebrate abundance), provide little information regarding size-class and age structure, growth rates, and relative importance of individual taxa or functional groups. Biomass, and particularly production estimates, allow for more comprehensive analysis of these important parameters, giving a more complete picture of community dynamics during disturbance and subsequent recovery. Despite this, the majority of lotic system recovery studies to this date have employed some form of abundance measure, including the occasional use of presence–absence information alone, for examination of recovery processes (see reviews by Niemi et al. 1990; Yount and Niemi 1990). However, abundance data in the form of biotic indices such as the North Carolina Biotic Index (Lenat 1993) and EPT (Ephemeroptera, Plecoptera, Trichoptera index) have recently been shown to depict changes in stream ecosystem processes associated with anthropogenic disturbance and subsequent recovery (Wallace et al. 1996).

Table 7. Average annual standing crop (g AFDM/m^2) of leaf litter, total coarse particulate organic matter (CPOM), and total fine particulate organic matter (FPOM) in the two major substrate types of catchments 54 and 55, and habitat-weighted values during 1985 (catchment 54 pretreatment), 1986 (catchment 54 treatment), 1989 (catchment 54 first-year recovery), and 1990 (catchment 54 second-year recovery).

	Catchment 54				Catchment 55			
	1985	1986	1989	1990	1985	1986	1989	1990
Mixed substrates								
Leaves	245.0*a*	315.4*a*	272.0*a*	98.4*b*	146.5*a*	217.1*a*	168.5*a*	62.5*b*
Total CPOM	1183.4*a*	1050.3*a*	1045.2*a*	750.5*b*	801.7*a*	883.9*a*	770.4*a*	359.9*b*
Total FPOM	784.1*ac*	1020.3*ab*	1223.4*b*	596.2*c*	517.7*ac*	683.5*b*	480.1*ab*	322.5*c*
Bedrock outcrop								
Leaves	9.7*a*	24.2*ab*	35.5*b*	18.3*ab*	6.5*a*	43.7*b*	12.4*ab*	18.8*ab*
Total CPOM	27.3*a*	44.0*a*	58.9*a*	34.7*a*	23.2*a*	73.9*a*	34.8*a*	40.7*a*
Total FPOM	30.8*a*	23.3*a*	43.8*b*	29.4*a*	23.5*a*	32.4*ab*	45.2*b*	28.6*ab*
Habitat weighted								
Leaves	162.6	213.5	189.2	70.4	128.3	194.6	148.2	56.8
Total CPOM	775.9	854.8	700.0	500.0	699.3	777.4	674.8	318.4
Total FPOM	518.6	671.4	810.5	397.8	452.7	598.9	423.6	284.3

Note: Values within a category in each stream that are followed by the same letter are not significantly different ($p < 0.05$, Kruskall–Wallis ANOVA on ranks and Dunn's test). Data for 1985 and 1986 are from Lugthart and Wallace (1992).

Estimates of production from C54 and C55 during this study are similar to those reported for the same streams (except during insecticide treatments) in previous studies (Lugthart and Wallace 1992), other small streams at Coweeta (Huryn and Wallace 1987*b*), and similar sized streams in other regions (Krueger and Waters 1983; Iverson 1988; Gaines et al. 1992). Our production estimates, as with other studies conducted in small streams, are substantially lower (per square metre) than many of those reported for larger streams and rivers, where production estimates over 40 times greater than those of C54 and C55 during this study have been reported (Voshell 1985; see Benke 1993 for review).

Physical factors influencing recovery

Record high precipitation during the 1989–1990 recovery period resulted in increased wetted areas of both streams (Wallace et al. 1991*a*). Annual estimates of wetted areas from drought (1986) to wet (1989) years increased by ca. 30 and 20% in C54 and C55, respectively. During this period, invertebrate abundance, biomass, and production in C54 increased, despite coinciding decreases in the reference stream as a result of a dilution effect. Areal estimates of production in C55 decreased by ca. 12% from 1986 to 1989. Thus, a 20% increase in the wetted area of C55 resulted in a 12% decline in areal production during this period. Wetted area estimates were not available for the subsequent 1990 wet year, but production further declined in C55 from 1989 to 1990 by ca. 20%. Although areal estimates in C55 decreased during 1989 and 1990, C54 values increased and surpassed those of C55. Thus, recovery processes in C54 were overriding the influence of 2 extremely wet years, and our areal production estimates during recovery may be underestimates of up to 18% during 1989, and more in 1990, on the basis of relationships observed in the C55 reference stream.

Table 8. Results of linear regressions performed on coarse particulate organic matter (CPOM) standing stocks (x) versus shredder biomass (y). Data were log transformed prior to analysis and are from individual benthic samples collected in catchments 54 and 55 during 1985 (catchment 54 pretreatment), 1986 (catchment 54 treatment), 1989 (catchment 54 first-year recovery), and 1990 (second-year recovery).

Stream and year	n	slope	r
Catchment 54			
1985	79	0.603	0.691*
1986	47	0.001	0.208
1989	77	0.438	0.394*
1990	84	0.589	0.572*
Catchment 55			
1985	77	0.557	0.696*
1986	49	0.591	0.704*
1989	49	0.451	0.540*
1990	42	0.655	0.515*

Note: Data for 1985 and 1986 are from Lugthart and Wallace (1992).

*Significant at $p < 0.001$.

Results of cluster analysis performed on production of all invertebrate taxa (Fig. 2A) further demonstrate the importance of hydrologic factors during this study. Aside from similarities in C54 data between 1989 and the 1986 treatment year, the two major clusters formed reflect drought and wet conditions during 1985–1986 and 1989–1990, respectively. As a result, the invertebrate community in

C54 during the second recovery year was apparently less similar to that of the pretreatment year than the treatment year or the first year of recovery. Thus, strong physical influences in the form of hydrologic extremes were apparently acting on the invertebrate communities of both streams throughout this study, and may have influenced recovery and (or) our interpretation of recovery processes. However, cluster analysis performed on functional group production showed no pattern of wet–dry years (Fig. 2B), suggesting that hydrologic fluctuations in these streams act on specific taxa rather than functional groups.

Record high precipitation during the 2 recovery years may also have indirectly affected invertebrate communities in both streams by altering organic matter dynamics. Significantly lower benthic leaf litter and total CPOM standing stocks in both streams during 1990 (Table 7) were a result of higher export and lateral deposition of this material during periods of unusually high discharge (Wallace et al. 1995). The influence of this benthic CPOM reduction on invertebrate recovery processes in C54 is not clear, but conceivably may have limited shredder recolonization (e.g., Richardson 1991; Dobson and Hildrew 1992), compounding the possibility of our underestimating invertebrate production during recovery of C54. These results suggest that our observations may be underestimates of what might be observed during normal years and underscore the importance of using a reference system during the same time frame as the study system. As demonstrated here, yearly comparisons within one system are subject to annual environmental variability that may confound other processes.

Differences in the macroinvertebrate communities of the two major substrate types observed during this study (see Table 2) are consistent with observations made by others (Gurtz and Wallace 1984; Huryn and Wallace 1987*b*; Lugthart and Wallace 1992; Whiles and Wallace 1992) and reflect physical differences between these habitats. More retentive and heterogeneous mixed substrate habitats of Coweeta streams are codominated by collector–gatherers, predators, and shredders. Bedrock outcrop habitats, which are characteristically areas of higher entrainment, are favored by collector–filterers and some shredder taxa. Appropriate groups recolonized mixed substrate habitats in C54 by the second recovery year. However, following initial recolonization of bedrock outcrops in C54, many important collector–filterer taxa (e.g., *Parapsyche cardis* (Trichoptera: Hydropsychidae) decreased by 1990 (see Appendix). In contrast, some taxa of other functional groups, such as the collector–gatherer stonefly *Amphinemura wui* and the scraping caddisfly *Neophylas mitchelli*, which were relatively unimportant on C54 bedrock outcrops before treatment (Lugthart and Wallace 1992) and during 1989 (see Appendix), were amongst the most productive taxa on bedrock outcrop habitats in C54 during 1990. Thus, C54 bedrock outcrop communities appeared initially to recover rapidly, but displayed marked annual variability over the 2 recovery years.

Other studies at Coweeta have noted differences in the effect of disturbance on bedrock outcrop and mixed substrate habitats. Gurtz and Wallace (1984) reported that bedrock outcrop habitats in a stream subjected to clear-cutting were more resistant to sedimentation than other substrates, and suggested that physical stability of this habitat resulted in biological stability. In contrast, Lugthart and Wallace (1992) found the bedrock outcrop habitat to be more sensitive to drought disturbance than mixed substrates. Results of the current study suggest that the invertebrate community of bedrock outcrops in C54 may be resilient to an insecticide disturbance but subject to considerable annual fluctuations in community structure.

Functional and taxonomic recovery patterns

Although insect and noninsect production levels in C54 during 1990 were both similar to C54 pretreatment values, C54 noninsect biomass during 1990 was approximately one third that of the pretreatment value. This is entirely a result of the loss of crayfish in C54. Although crayfish can contribute substantially to biomass estimates, their low abundances and slow growth rates in these streams do not contribute significantly to abundance or production estimates (Huryn and Wallace 1987*a*). Crayfish have limited dispersal abilities and failed to recolonize C54 during the course of this study. In contrast, most insect taxa were capable of recolonizing via aerial adults, and many smaller noninsect taxa have short life cycles and resistant life stages that enabled them to persist in C54 during treatment (Lugthart and Wallace 1992).

In most cases, production values in C54 were similar to those in the reference stream by 1990. However, differences in functional group P/B ratios and voltinism of inhabitant taxa indicate lingering taxonomic differences (see Tables 4 and 6). High scraper P/B ratios in C54 during 1989 reflect the relative importance of smaller, faster growing scrapers (e.g., *Baetis* spp. (Ephemeroptera: Baetidae)), and the relative scarcity of larger and (or) longer lived scrapers (e.g., *Stenonema* spp. (Ephemeroptera: Heptageniidae) and *Neophylax mitchelli*). Similarly, relatively high shredder P/B ratios in C54 during both 1989 and 1990 reflect the predominance of small univoltine shredders such as *Lepidostoma* spp., and reduced numbers of larger, semivoltine shredders (e.g., *Tallaperla* spp. and *Fattigia pele* (Trichoptera: Sericostomatidae)).

Higher shredder P/B ratios in C54 compared with C55 also reflect differences in the age structure of longer lived taxa between the two streams. Macroinvertebrate shredders are a vital component of detritus dynamics and energy flow in Coweeta headwater streams (Wallace et al. 1982, 1986; Cuffney et al. 1984, 1990), as well as forested headwater streams in general (e.g., Vannote et al. 1980). Peltoperlid stoneflies, which are semivoltine, have been shown to be one of the most abundant, productive, and thus important shredders in small undisturbed Coweeta streams (O'Hop et al. 1984; Lugthart and Wallace 1992). However, during most of the 2-year recovery period, only one *Tallaperla* spp. cohort was well represented in C54. *Lepidostoma* spp., smaller, univoltine trichopteran shredders, contributed greatly to recovery of shredder abundance (see Fig. 6) and production (see Appendix) in C54. Thus, although shredder production in C54 was 0.71 g AFDM·m^{-2}·year^{-1} higher than that in C55 by 1990, the average individual body size of shredders in C54 (0.30 mg AFDM) was less than half that of shredders in the reference stream (0.63 mg AFDM), and

habitat-weighted annual average shredder biomass was substantially less in C54 (0.374 g/m^2) than in C55 (0.572 g/m^2).

Differences in the C54 shredder community undoubtedly influenced ecosystem-level processes in C54. Despite low shredder biomass, processing of both red maple and rhododendron litter in C54 was substantially faster than in C54 before treatment and in C55 by 1990 (Chung et al. 1993). Additionally, Whiles et al. (1993) linked high densities of *Lepidostoma* spp. with unusually fast litter processing rates during recovery periods of disturbed Coweeta streams. Thus, although smaller in size, *Lepidostoma* spp. were extremely important in C54 during recovery. Chung (1992) noted that litter decomposition dynamics in C54 during recovery more closely tracked dynamics of trichopteran shredders in litterbags than other shredders. Differences in shredder production between C54 (2.77 g AFDM·m^{-2}·year^{-1}) and the reference stream (2.06 g AFDM·m^{-2}·year^{-1}), when converted to consumption rates using assimilation efficiency and net production efficiency values from McDiffett (1970) and Perry et al. (1987), suggest that shredders in C54 consumed 14 g AFDM·m^{-2}·year^{-1} more leaf litter than those in the reference stream during 1990. As a result, the processing time of CPOM inputs into C54 during 1990 was probably shorter than that of similar undisturbed streams at Coweeta, and particle generation may have been enhanced. In agreement, Wallace et al. (1991*a*) observed a rapid recovery of seston concentration in C54.

The positive correlation of shredder biomass and CPOM in C55 and C54 before treatment suggests that, in undisturbed Coweeta streams, CPOM is a limiting resource (see Table 8). Others have also recently demonstrated that CPOM can be a limiting resource to shredders (Richardson 1991; Dobson and Hildrew 1992). However, during treatment of C54, shredder densities were severely reduced and large accumulations of unprocessed leaf litter were present (Wallace et al. 1995). As a result, CPOM was apparently no longer limiting during treatment, as no significant relationship between shredder biomass and CPOM was evident. With gradual recolonization of shredder taxa, regression slopes and correlation coefficients of shredder biomass and CPOM in C54 increased, reflecting restoration of this relationship over the 2 years following treatment.

In a previous study that examined recolonization of invertebrates in litterbags following a similar, but shorter (1 year) disturbance, Wallace et al. (1986) observed a shredder facilitation effect, whereby recovery of particle-feeding collectors in litterbags followed that of shredders. During our study, no such effect was observed for the benthos as a whole. Both collector–filterers and collector–gatherers recovered rapidly and appeared to precede shredders as a group (see Fig. 3 and Table 3). This apparent lack of a facilitation effect was most likely a result of physical factors associated with discharge and longer treatment of C54. The high standing stock of FPOM in C54 during the first recovery year (see Table 7) was most likely a result of reduced export and higher retention of this material during the preceding 1986–1988 drought. Large accumulations of unprocessed leaf litter in C54 by the third year of treatment (1988) (Wallace et al. 1995) probably further contributed to retention of FPOM. Additionally, high precipitation and discharge during the first recovery year resulted in expanded stream channels, and undoubtedly enhanced inputs of previously unavailable particulate organic matter lateral to the stream channel, as well as entrainment of previously accumulated FPOM. Wallace et al. (1991*a*) observed seston concentrations shortly after cessation of treatments in C54 similar to those of undisturbed Coweeta streams. Thus, regardless of shredder activity, the physical characteristics of the treatment and recovery periods in C54 resulted in conditions that favored collector–gatherers and filterers. Additionally, rapid recolonization of *Lepidostoma* spp. in C54 may have further enhanced FPOM generation during recovery of C54, despite reduced production of other shredders.

Changes in C54 during the recovery period are clearly a result of a shift from seasonal anthropogenic disturbance to no anthropogenic disturbance. Lugthart and Wallace (1992) demonstrated that during treatment of C54, small-bodied taxa with fast developmental rates dominated the invertebrate community. Heckman (1981) observed similar increases in small-bodied taxa with faster developmental periods in orchard streams exposed to insecticide. Similarly, during the first recovery year of C54, Whiles and Wallace (1992) observed relatively high abundances and biomass of many smaller taxa, while those of many larger taxa with longer life cycles, particularly semivoltine taxa, were still reduced. Results of the current study show a 2-year recovery pattern of decreases in small, multivoltine taxa (e.g., polyvoltine and bivoltine insect taxa and most noninsect taxa) and increases in larger, univoltine and semivoltine taxa.

Predators showed one of the largest increases in production of all functional groups from treatment through recovery of C54, resulting in ca. 25% higher predator production during 1990 than before treatment (see Fig. 3). Decreases in many small-bodied collector–gatherer taxa (e.g., Copepoda (see Fig. 4)) correspond with increased predator production, suggesting that invertebrate predators are an important regulator of community structure in Coweeta headwater streams. Wallace et al. (1987) found that an insecticide-tolerant predator in C54, *Lanthus vernalis* Carle (Odonata: Gomphidae), readily switched to smaller noninsect prey items during treatment, when larger insect prey were scarce. High densities of small collector–gatherer taxa following treatments in C54 undoubtedly represented a rich food resource for recolonizing predators. As recovery proceeded, it is likely that predation pressure reduced densities of Copepoda and other small collector–gatherers to levels similar to those in the reference stream, and in C54 during the pretreatment period.

Major factors influencing recovery of C54 during this study were characteristics of the life cycles of individual taxa, especially life-cycle length. Taxa with shorter life cycles and (or) extended flight periods were capable of more rapid recolonization than those with longer life cycles and (or) shorter flight periods. These observations agree closely with those of other recovery studies (e.g., Ide 1967; Gray 1981; Fisher et al. 1982; Molles 1985). Because C54 was treated from the spring seep down to a gauging flume, upstream or downstream source areas for larval colonists were not present. Thus, recolonization was primarily limited to ovipositing adults (Wallace et al. 1991*b*). The close

proximity of numerous source areas (undisturbed streams) for colonists at Coweeta was undoubtedly an important factor contributing to rapid recovery of many taxa.

After 2 years of recovery, overall community and functional structure in C54 was similar to that in the reference stream, as well as in C54 in the pretreatment period. However, subtle taxonomic and developmental stage differences persisted, because of a positive relationship between life-cycle length and recovery time. A similar recovery pattern (functional recovery preceding taxonomic recovery) was observed in a similar study at Coweeta in which recovery of macroinvertebrates in litterbags was examined (Wallace et al. 1986). Many of the differences that persist in C54 after 2 years are associated with shredder taxa, suggesting that lingering effects on energy flow are possible. However, rapid recolonization of some taxa (e.g., *Lepidostoma* spp.) appears to have compensated for the slow recovery of others, demonstrating that some taxa with high vagility may play disproportionately important roles during recovery. Additionally, changes in the reference stream during the course of this study indicate that the magnitude of the recovery sequence we observed may have been influenced by prevailing physical conditions. Thus, recovery patterns in C54 during a period of normal environmental conditions may have differed.

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Appendix

Annual mean abundance (Ab; individuals/m^2), biomass (Bi; mg AFDM/m^2), and production (Pr; mg AFDM·m^{-2}·$year^{-1}$) of major invertebrate taxa in mixed substrates and bedrock outcrop habitats of catchments 54 and 55 (C54 and C55) during 1989 (year 1) and 1990 (year 2).

				Mixed substrates			Bedrock outcrop		
Taxon	Order[a]	Stream	Year	Ab	Bi	Pr	Ab	Bi	Pr
Scrapers									
Baetis spp.	E	C54	1	35	6	188	44	2	31
		C54	2	17	1	26	138	5	147
		C55	1	0	0	0	0	0	0
		C55	2	18	3	44	76	2	74
Epeorus sp.	E	C54	1	<1	<1	1	7	4	70
		C54	2	1	<1	1	27	27	131
		C55	1	0	0	0	16	4	23
		C55	2	0	0	0	5	5	15
Stenonema sp.	E	C54	1	14	4	23	1	1	14
		C54	2	26	41	166	5	1	13
		C55	1	26	19	110	12	<1	5
		C55	2	27	16	91	2	1	5
Hydroptila coweetensis	T	C54	1	0	0	0	36	1	5
		C54	2	0	0	0	27	5	25
		C55	1	0	0	0	125	3	31
		C55	2	0	0	0	31	5	26
Neophylax mitchelli	T	C54	1	0	0	0	5	1	<1
		C54	2	11	1	12	109	17	232
		C55	1	0	0	0	14	1	33
		C55	2	13	<1	3	5	3	13
Ectopria thoracica	C	C54	1	4	<1	5	13	8	58
		C54	2	4	1	7	7	7	35
		C55	1	0	0	0	20	10	35
		C55	2	8	5	39	19	22	116
Other taxa[b]		C54	1	96	5	23	0	0	0
		C54	2	0	0	0	1	<1	<1
		C55	1	120	5	17	41	3	7
		C55	2	343	12	48	69	3	12
Total scrapers		C54	1	149	11	240	107	17	178
		C54	2	57	43	212	314	61	584
		C55	1	146	24	127	229	21	134
		C55	2	409	36	225	207	41	262
Shredders									
Leuctra spp.	P	C54	1	157	13	38	21	<1	2
		C54	2	181	28	122	40	1	8
		C55	1	640	18	158	59	1	13
		C55	2	369	8	51	133	9	36
Tallaperla spp.	P	C54	1	18	1	15	82	42	218
		C54	2	23	37	142	153	67	436
		C55	1	149	73	209	1 130	192	988
		C55	2	243	48	324	1 071	200	1316
Lepidostoma spp.	T	C54	1	332	87	1036	182	27	445
		C54	2	1 267	111	1322	279	18	220
		C55	1	155	25	208	102	6	68
		C55	2	114	9	106	55	9	77
Pycnopsyche spp.	T	C54	1	47	10	331	14	1	12
		C54	2	32	21	94	8	11	24
		C55	1	213	98	868	39	4	54
		C55	2	19	35	192	0	0	0

Appendix (*continued*).

Taxon	Order[a]	Stream	Year	Mixed substrates			Bedrock outcrop		
				Ab	Bi	Pr	Ab	Bi	Pr
Molophilus spp.	D	C54	1	165	29	160	0	0	0
		C54	2	78	34	138	0	0	0
		C55	1	191	25	172	16	1	5
		C55	2	38	6	22	0	0	0
Tipula spp.	D	C54	1	37	182	929	44	57	353
		C54	2	49	238	1686	16	67	530
		C55	1	23	287	1218	16	23	254
		C55	2	11	302	971	17	80	613
Decapoda		C54	1	0	0	0	0	0	0
		C54	2	0	0	0	0	0	0
		C55	1	1	206	140	0	0	0
		C55	2	2	61	35	0	0	0
Other taxa[c]		C54	1	3	3	16	0	0	0
		C54	2	6	16	78	4	2	5
		C55	1	43	67	121	2	<1	<1
		C55	2	65	147	364	0	0	0
Total shredders		C54	1	759	325	2524	344	127	1030
		C54	2	1 636	485	3581	499	167	1222
		C55	1	1 416	800	3094	1 366	227	1382
		C55	2	859	617	2065	1 275	297	2042
Collector–gatherers									
Paraleptophlebia spp.	E	C54	1	90	2	15	10	<1	1
		C54	2	43	3	20	10	1	7
		C55	1	247	22	112	22	3	6
		C55	2	129	10	60	12	1	3
Serratella sp.	E	C54	1	15	<1	5	126	25	418
		C54	2	123	3	51	360	75	430
		C55	1	23	4	18	133	23	124
		C55	2	24	3	21	33	15	75
Amphinemura wui	P	C54	1	1	0	5	152	20	145
		C54	2	123	13	104	1 008	72	841
		C55	1	19	1	17	477	37	361
		C55	2	88	11	92	735	60	547
Soyedina carolinensis	P	C54	1	103	11	59	64	10	163
		C54	2	91	7	75	66	14	57
		C55	1	17	4	11	4	1	4
		C55	2	31	<1	6	14	2	12
Chironomidae	D	C54	1	18 183	184	1900	6 361	41	574
		C54	2	12 951	86	1461	2 898	12	211
		C55	1	13 793	71	1178	20 093	92	1322
		C55	2	14 832	65	1135	7 852	32	509
Copepoda		C54	1	49 765	50	896	10 287	10	185
		C54	2	35 906	36	646	2 750	3	50
		C55	1	11 250	11	203	2 977	3	54
		C55	2	8 460	8	152	1 420	1	26
Nematoda		C54	1	10 840	8	41	830	1	3
		C54	2	9 639	7	37	239	<1	1
		C55	1	6 843	5	26	463	<1	2
		C55	2	4 264	3	16	354	<1	1
Oligochaeta		C54	1	9 538	493	2465	3 588	35	174
		C54	2	8 365	280	1398	788	20	99
		C55	1	3 650	107	647	942	8	37
		C55	2	3 763	103	514	561	4	19

Appendix (*continued*).

Taxon	Order[a]	Stream	Year	Mixed substrates			Bedrock outcrop		
				Ab	Bi	Pr	Ab	Bi	Pr
Other taxa[d]		C54	1	2 193	7	56	35	<1	1
		C54	2	461	6	29	13	1	2
		C55	1	153	113	95	26	<1	2
		C55	2	139	39	52	51	1	3
Total collector-gatherers		C54	1	90 728	755	5442	21 452	142	1665
		C54	2	67 703	441	3822	8 132	197	1698
		C55	1	35 995	339	2307	25 138	168	1912
		C55	2	31 730	243	2048	11 031	116	1195
Collector–filterers									
Diplectrona metaqui	T	C54	1	7	16	63	20	18	251
		C54	2	11	17	76	5	15	35
		C55	1	0	0	0	31	19	173
		C55	2	0	0	0	2	2	4
Diplectrona modesta	T	C54	1	118	28	383	82	12	93
		C54	2	362	73	602	129	14	122
		C55	1	76	25	207	276	59	672
		C55	2	224	79	525	534	64	637
Parapsyche cardis	T	C54	1	3	<1	4	88	72	557
		C54	2	1	1	9	42	33	204
		C55	1	2	1	<1	270	172	1477
		C55	2	4	11	58	284	151	1015
Wormaldia moesta	T	C54	1	34	4	35	34	10	190
		C54	2	18	5	37	60	5	212
		C55	1	26	6	85	63	23	121
		C55	2	26	7	128	31	4	39
Simuliidae	D	C54	1	30	2	17	168	8	58
		C54	2	0	0	0	26	<1	4
		C55	1	0	0	0	170	12	334
		C55	2	0	0	0	0	0	28
Other taxa[e]		C54	1	24	0	4	10	1	3
		C54	2	14	3	7	30	1	3
		C55	1	53	6	2	22	<1	23
		C55	2	2	<1	<1	31	<1	2
Total collector-filterers		C54	1	215	52	506	402	121	1152
		C54	2	405	98	731	292	67	580
		C55	1	156	37	294	833	285	2800
		C55	2	256	97	712	883	221	1724
Predators									
Cordulegaster sp.	O	C54	1	47	204	321	0	0	0
		C54	2	37	431	706	0	0	0
		C55	1	22	73	101	0	0	0
		C55	2	20	77	104	0	0	0
Lanthus vernalis	O	C54	1	83	302	842	3	24	197
		C54	2	109	300	1409	2	18	63
		C55	1	31	85	195	4	75	47
		C55	2	38	44	186	2	1	6
Beloneuria spp.	P	C54	1	0	0	0	0	0	0
		C54	2	71	<1	4	14	<1	1
		C55	1	19	45	88	59	52	171
		C55	2	74	4	22	85	25	60
Isoperla spp.	P	C54	1	16	1	18	13	4	62
		C54	2	25	6	35	94	10	83
		C55	1	63	10	34	31	3	15
		C55	2	20	2	11	19	2	24

Appendix (*continued*).

Taxon	Order[a]	Stream	Year	Mixed substrates			Bedrock outcrop		
				Ab	Bi	Pr	Ab	Bi	Pr
Sweltsa lateralis	P	C54	1	8	<1	1	1	<1	<1
		C54	2	26	2	10	0	0	0
		C55	1	78	12	30	4	1	2
		C55	2	79	14	34	0	0	0
Rhyacophila spp.	T	C54	1	4	<1	7	15	11	86
		C54	2	14	1	5	55	26	103
		C55	1	55	41	132	127	78	214
		C55	2	117	6	64	88	34	145
Ceratopogonidae	D	C54	1	4 107	75	392	118	2	15
		C54	2	3 434	224	1522	252	13	96
		C55	1	2 414	47	1338	162	3	68
		C55	2	1 384	97	446	246	9	37
Dicranota spp.	D	C54	1	43	3	58	51	1	25
		C54	2	119	10	56	109	4	28
		C55	1	18	<1	1	167	6	31
		C55	2	25	3	16	93	4	24
Dolichopodidae	D	C54	1	60	11	443	0	0	0
		C54	2	32	7	28	0	0	0
		C55	1	2	2	5	0	0	0
		C55	2	2	4	17	0	0	0
Glutops sp.	D	C54	1	0	0	0	0	0	0
		C54	2	55	35	216	0	0	0
		C55	1	2	20	17	0	0	0
		C55	2	0	0	0	0	0	0
Hexatoma spp.	D	C54	1	135	20	84	1	1	2
		C54	2	124	49	144	2	1	5
		C55	1	315	74	378	6	1	5
		C55	2	239	82	332	5	1	5
Pedicia spp.	D	C54	1	5	12	104	1	5	72
		C54	2	42	126	905	0	0	0
		C55	1	36	2	11	12	1	2
		C55	2	2	1	5	2	<1	2
Pseudolimnophila spp.	D	C54	1	191	60	234	10	4	15
		C54	2	206	70	284	60	9	28
		C55	1	45	25	93	2	<1	1
		C55	2	22	8	73	0	0	0
Tanypodinae	D	C54	1	3 172	52	244	243	4	19
		C54	2	945	10	67	160	1	9
		C55	1	644	5	366	698	4	283
		C55	2	342	2	17	308	1	9
Acari		C54	1	7 014	19	93	2 949	8	39
		C54	2	4 366	12	58	2 217	6	30
		C55	1	1 457	4	17	1 992	5	25
		C55	2	1 542	4	21	1 605	4	21
Turbellaria		C54	1	459	15	76	863	29	143
		C54	2	106	5	23	153	6	31
		C55	1	110	5	22	248	10	48
		C55	2	45	2	10	79	1	7
Other taxa[f]		C54	1	57	6	25	30	7	22
		C54	2	191	33	218	91	1	6
		C55	1	133	136	380	387	19	86
		C55	2	81	117	471	135	5	26

Appendix (*concluded*).

Taxon	Order[a]	Stream	Year	Mixed substrates			Bedrock outcrop		
				Ab	Bi	Pr	Ab	Bi	Pr
Total predators		C54	1	15 401	779	2941	4 298	98	698
		C54	2	9 902	1319	5688	3 208	97	482
		C55	1	5 443	587	3208	3 899	258	998
		C55	2	4 032	467	1828	2 666	89	366

Note: Biomass estimates were obtained using length–weight regressions. Production of most insect taxa was estimated with the size–frequency method (Hamilton 1969) corrected for the cohort production interval (Benke 1979), except for non-tanypodine Chironomidae (community-level method (Huryn 1990)). Production of noninsects and uncommon insect taxa was estimated by multiplying mean annual biomass by literature P/B values (Waters 1977) or P/B values from other studies (see text).

[a]E, Ephemeroptera; P, Plecoptera; O, Odonata; T, Trichoptera; D, Diptera; C, Coleoptera.

[b]*Stenacron* sp., *Thaumalea thornburghae*, *Optioservus immunis*, *Oulimnus latiusculus*.

[c]*Fattigia pele*, *Psilotreta* sp., *Theliopsyche* sp., *Leptotarsus* sp., *Limonia* sp., *Anchytarsus bicolor*.

[d]*Habrophlebia vibrans*, *Lype diversa*, Nymphomyiidae, *Ormosia* sp., *Sciara* sp., Cladocera, Decapoda, Ostracoda.

[e]*Dolophilodes* sp., *Polycentropus* sp., *Dixa* sp.

[f]*Pseudogoera singularis*, Empididae, nr. *Pedicia*, Decapoda.

Larval fish drift in the Valley River, Manitoba: influence of abiotic and biotic factors, and relationships with future year-class strengths

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Abstract: We examined patterns in the spring drift of larval fishes in the Valley River, Manitoba. White sucker (*Catostomus commersoni*) and walleye (*Stizostedion vitreum*) were the most common species. The median date of larval drift for walleye was significantly earlier than for all other species. Capture rates of all species were significantly greater during the night than during the day. Larval abundance was positively related to river discharge in the 35 d preceding the median date of larval drift. Larval abundance was also positively related to mature female biomass in Dauphin Lake for walleye, but not for white sucker. Year-class strength of a cohort (catch per unit effort of 3- or 4-year-old fish) in Dauphin Lake was positively related to total larval drift of the same cohort (3 or 4 years earlier) in the Valley River for walleye, but not for white sucker. Our results suggest that the year-class strength of Dauphin Lake walleye is determined very early in the life history and that factors which enhance the natural production of walleye larvae will enhance walleye recruitment.

Résumé : Nous avons examiné le régime de dérive printanière des larves de poissons dans la rivière Valley, au Manitoba. Le meunier noir (*Catostomus commersoni*) et le doré (*Stizostedion vitreum*) étaient les espèces les plus communes. La date médiane de dérive des larves chez le doré était nettement antérieure à celle de toutes les autres espèces. Les taux de capture de toutes les espèces étaient nettement plus élevés pendant la nuit que pendant le jour. L'abondance des larves était positivement liée au débit de la rivière dans les 35 jours qui précédaient la date médiane de dérive; elle était aussi positivement liée à la biomasse de femelles matures dans le lac Dauphin pour le doré, mais pas pour le meunier noir. L'effectif de la classe d'âge d'une cohorte (prise par unité d'effort de poissons âgés de 3 ou 4 ans) dans le lac Dauphin était positivement liée à la dérive totale des larves de la même cohorte (3 ou 4 ans plus tôt) dans la rivière Valley pour le doré, mais pas pour le meunier noir. Nos résultats permettent de penser que l'effectif des classes d'âge du doré du lac Dauphin est déterminé très tôt dans le cycle biologique de ce poisson, et que les facteurs qui accroissent la production naturelle de larves de doré améliorent le recrutement de ce poisson.

[Traduit par la Rédaction]

Introduction

The Valley River is the largest of seven major tributaries entering Dauphin Lake in west-central Manitoba. Its drainage area comprises 40% (2870 km^2) of the total Dauphin Lake drainage basin (Chapman 1987). The Valley River drains the southern portion of the heavily forested Duck Mountains as well as the agriculturalized parkland between the Duck Mountains and Riding Mountain, and enters the northwest section of Dauphin Lake. It has undergone the least amount of physical alteration of all the major rivers flowing into Dauphin Lake (Chapman 1987). Because it contains the largest area of natural spawning sites of all the Dauphin Lake tributaries, conditions in the Valley River are believed to determine the spawning success of the riverine-spawning species of the lake.

Historically, Dauphin Lake supported a very productive commercial fishery, particularly for walleye, *Stizostedion vitreum* (Cunningham 1935; Gaboury 1985). However, annual yields declined drastically during the 1950s and 1960s and have remained at low levels (Gaboury 1985). Overexploitation and the destruction of spawning habitat in the surrounding drainage basin were considered the major

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causes of this decline (Gaboury 1985). The Manitoba Department of Natural Resources, in cooperation with the Canada Department of Fisheries and Oceans, initiated a study of the Dauphin Lake fishery in 1982 to examine the dynamics of its fish populations, determine which factors were most important in the collapse, and assess the feasibility of various rehabilitation plans such as improving degraded spawning habitat along inflowing rivers (Newbury and Gaboury 1988, 1993) and stocking walleye larvae (Mathias et al. 1992).

As part of the Dauphin Lake study, the Manitoba Department of Natural Resources monitored the spring drift of larval fishes in the Valley River each year starting in 1982. Long-term studies of interannual variation in larval fish production in similar systems are generally lacking. We had three main objectives in carrying out this study. First, we wished to examine the species composition, timing, pattern, and interannual variation of spring larval fish drift in the Valley River. Second, we wanted to examine some of the factors that may influence the abundance of drifting larval fishes, particularly spring water temperature, water flow, and the abundance of spawning-age females. Finally, we wanted to examine how the abundance of larval fish related to future year-class strength.

Materials and methods

Data collection

Walleye spawning sites on the Valley River were identified between 20 and 70 km upstream from Dauphin Lake (Fig. 1). A water control structure at Gilbert Plains (95 km upstream from Dauphin Lake) restricts further upstream fish migration except during periods of high flow. Complete descriptions of the Valley River and Dauphin Lake environments are provided by Chapman (1987) and Babaluk and Friesen (1990), respectively. Larval drift was monitored from 1982 to 1992 in a section of the Valley River 16–18 km upstream from Dauphin Lake (Fig. 1).

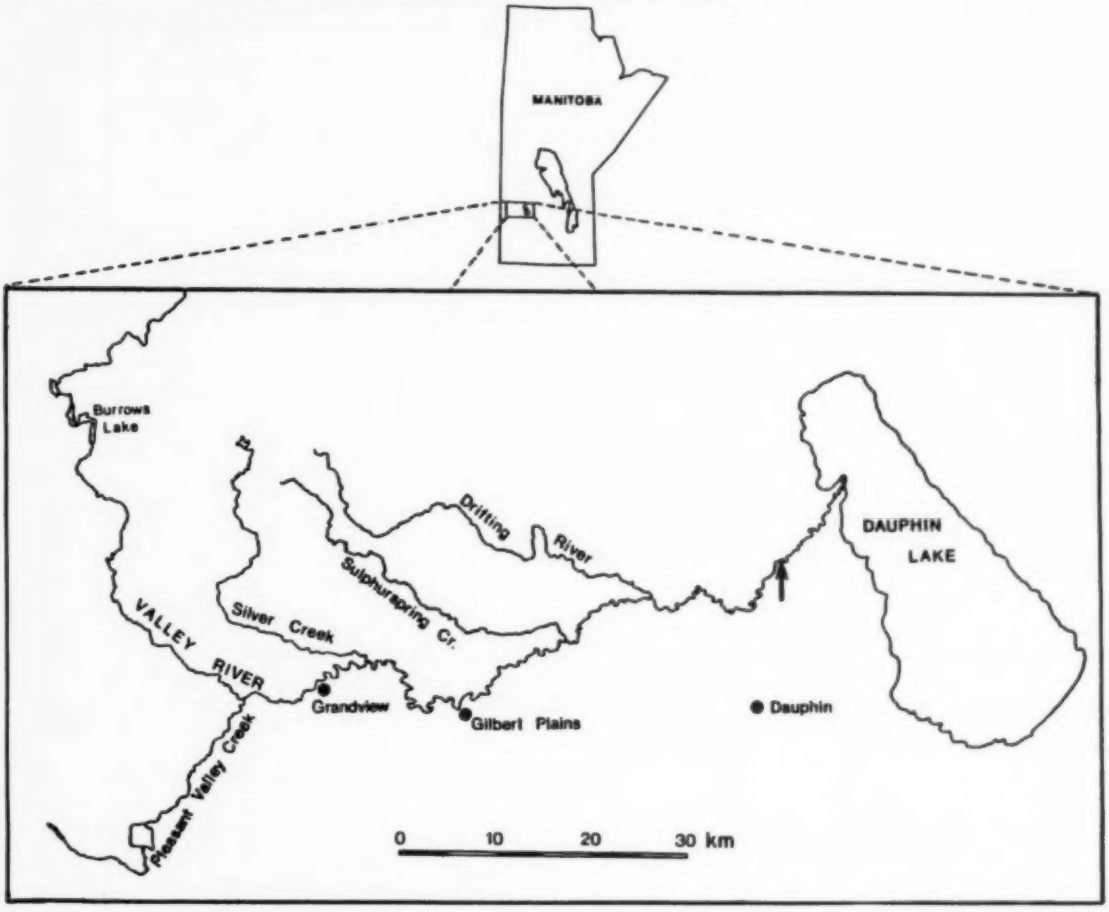
Larvae were sampled with drift bombs modified from the design of Burton and Flannagan (1976). Bombs consisted of a 750-μm Nitex® net (length, 1 m) mounted in the rear of an aluminum mouth-reducing cone that was square in cross section (front opening, 15.2 × 15.2 cm; mouth area, 0.0232 m^2; rear opening, 25.4 × 25.4 cm; length, 25 cm). A removable, 1-L, wide-mouthed Nalgene® collection bottle with two 750-μm Nitex windows was attached to the cod end of the net with a hose clamp. The bomb sampler was attached to steel posts driven into the substrate. In 2 years, 1988 and 1990, high flows necessitated attaching the bomb to the base of a floating raft. In all cases, the sampler was placed in the thalweg at ~20 cm below the water surface. We used two drift bombs in 1984, 1987, 1988, and 1989, and a single drift bomb in all other years.

We deployed drift samplers each spring several days prior to the predicted onset of walleye hatching. This date was predicted by estimating from water temperature the time of first spawning and the duration of incubation. We assumed that spawning started when the river temperature reached 7°C (Colby et al. 1979), and we predicted the time from spawning to hatching using an empirical relationship between incubation time and temperature (W.L. Hartman, unpublished data, cited in Colby et al. 1979). Generally, the drift samplers were removed 2–7 d after daily catches of walleye larvae had diminished to $0 \cdot d^{-1}$. However, in 1992, walleye larvae drifted over an unusually long time and some larvae were still being captured on the final sampling date. The collection bottle was emptied daily at 09:00 and the sample fixed in 5% Formalin. On several dates from 1988 to 1992, the collection bottle was emptied twice daily at 09:00 and 18:00. These samples were referred to as night and day samples, respectively. Preserved larvae were later counted, and identified according to Auer (1982). Counts were based on total samples except on several dates in 1990 when catches were very high (>20 000 larvae/sample) and counts were based on subsamples. Walleye, white sucker (*Catostomus commersoni*), and quillback (*Carpiodes cyprinus*) larvae were counted separately in all years (except 1984 and 1986 for quillback). Remaining larvae were grouped as other Percidae (primarily yellow perch, *Perca flavescens*) or other species.

We estimated total drift during a sample period by dividing the sample count by the estimated percentage of the total flow sampled. The percentage of the total flow sampled was estimated as the ratio of the flow rate through the bomb (net flow; cubic metres per second) to the mean daily flow for the Valley River near our study site (stream flow (SF). Net flows were estimated as the product of the aperture area and water velocity. Water velocity was measured at the bomb mouth with a Gurley (1982–1988) or Ott (1989–1992) current meter. Net flows were determined infrequently (three or four times per sampling season) from 1982 to 1988, but were determined daily from 1989 to 1992. Stream flows (mean daily discharge) were obtained from a series of annual data reports published by Environment Canada (e.g., Water Survey of Canada 1990). We developed an empirical relationship between the percentage of the total flow sampled (PS = 100(net flow/SF)) and SF to estimate PS for all sampling dates on which net flow was not determined directly ($\log_e PS = 0.061 - 0.628 \cdot \log_e SF$, $n = 91$, $R^2 = 0.82$). Catches and estimated total abundances of fish larvae were previously summarized in a series of manuscript reports for 1982 and 1983 (Gaboury 1985), and 1985–1988 (Cann 1990, 1991*a*, 1991*b*, 1992).

In several analyses we examined the relationships between larval fish drift, water temperature, water flow, and the abundance of adult and subadult fish stocks in Dauphin Lake. Mean daily water temperatures in the Valley River were estimated as the mean of maximum and minimum daily temperatures as recorded with a Ryan thermograph. Temperature was measured at the river bottom just downstream of our sampling site. Temperature was not monitored continuously in 1984 and 1992, and data from these years were excluded in any temperature-related analyses. We used measures of mean and minimum discharge over the 35 d preceding the median date of larval drift because we felt that this period covered the time necessary for upstream migration of adults, spawning, and egg incubation. Babaluk et al. (1992) provided data on the abundances, and age, size, and sex composition of adult and subadult walleye and white sucker populations in Dauphin Lake from 1984 to 1989. Quantitative data on adults and

Fig. 1. The Valley River drainage basin. Other Dauphin Lake tributaries are not shown. Solid circles indicate major population centres. Arrow indicates location where larval drift was monitored from 1982 to 1992.



subadults for other years and other species were not available. The catch per unit effort (CPUE, total biomass (kilograms) captured in 15 overnight gill-net sets during an autumn survey) for a given age-class was used as an index of year-class strength. The CPUE of mature females summed across all age categories was used as an index of spawning stock. The CPUE of mature females was estimated for each age-class as the product of mean individual wet weight, number of females captured, and the fraction that were mature. For walleye, we assumed that 50% of 5-year-old females and 100% of ≥6-year-old females were mature (Babaluk et al. 1993). For white sucker, we assumed that 25% of 4-year-old, 65% of 5-year-old, 75% of 6-year-old, and 90% of ≥7-year-old females were mature (J.A. Babaluk, Freshwater Institute, Winnipeg, Man., unpublished data).

Analyses

Statistical analyses were performed only on data of larvae identified to species. We tested the hypothesis that the timing of larval drift differed between species in two steps. First, we compared the median date of drift (the date when 50% of the total annual drift was captured) between species using ANOVA (GLM procedure, SAS Institute Inc. 1985) with years treated as blocks. Means were compared using the Tukey option of the GLM procedure (SAS Institute Inc. 1985). Second, we compared the discreteness of the drift period (i.e., large proportion of drift concentrated on few days versus drift spread out more evenly over many days) between species. In this analysis, we used the standard deviation of the date of drift to represent the spread in drift and compared species using block-designed ANOVA as above.

We conducted separate analyses for each species to test the hypothesis that more larvae were captured at night than during the day. We used data from all dates on which paired day and night samples were collected and on which at least one larva was captured during the day or night. Because night samples were collected over a longer duration than day samples, catches were first standardized by dividing the number of larvae by sampling duration (larvae per

Table 1. Summary of estimated total annual abundance (total, 1000s) and species composition of larval fish drifting downstream in the Valley River, Manitoba from 1982 to 1992 (WA, walleye; OP, other percids; WS, white sucker; QB, quillback; OS, other species).

		Species composition (%)					$\bar{Q}$		IT		Female SSB		YCS	
Year	Total	WA	OP	WS	QB	OS	WA	WS	WA	WS	WA	WS	WA	WS
1982	79	3.0	<0.1	50.8	46.2	<0.1	4.6	2.4	11	14	—	—	10	9
1983	55 099	0.8	0.9	96.0	2.3	<0.1	35.0	11.2	15	15	—	—	117	21
1984	14	17.5	<0.1	82.5	—	<0.1	2.5	2.5	—	—	8.9	30.1	1	20
1985	4 570	0.3	<0.1	99.6	<0.1	<0.1	19.2	14.8	9	13	5.7	20.4	46	1
1986	1 952	0.6	0.9	98.5	—	<0.1	20.0	20.7	12	14	13.9	57.8	28	0
1987	967	6.8	3.9	74.1	14.9	0.2	23.5	24.1	11	15	26.6	74.4	—	—
1988	1 048	9.7	4.2	76.9	6.0	3.1	20.0	23.1	13	14	34.8	48.5	—	—
1989	34	69.1	6.4	23.0	1.5	<0.1	1.5	1.4	10	15	47.4	37.2	—	—
1990	93 374	4.5	0.5	92.5	2.3	0.2	26.0	21.0	12	15	—	—	—	—
1991	165	12.1	30.6	34.3	4.0	19.0	2.2	1.5	15	18	—	—	—	—
1992	1 302	55.7	2.4	34.3	7.6	<0.1	18.4	18.1	—	—	—	—	—	—

Note: Quillback larvae were not separated from other species (OS) in 1984 and 1986. Variables used in analyses of larval drift abundance were mean discharge in the 35 d preceding the median drift date ($\bar{Q}$; $m^3 \cdot s^{-1}$), incubation time (IT; d), female spawning stock biomass (SSB, kg/15 gill-net nights), and future year-class strength (YCS; number of 3+ fish/15 gill-net nights). Year-class strength for a given year in the table represents catch per unit effort 3 years later. Sources of data and methods used to estimate variable values are described in the text.

hour). Day and night catches of larvae were compared using one-sided, paired-observation *t* tests (Steel and Torrie 1980). Catches were transformed by $\log_e(x + 1)$ to normalize the residuals. However, we were unable to normalize the residuals for the walleye data by transformation and therefore used the nonparametric Wilcoxon's signed rank test (Steel and Torrie 1980).

We examined the effect of water temperature indirectly by using a variable representing incubation time. Incubation time was estimated as the length of time required to attain a specified number of degree-days measured backwards from the median date of larval drift for a given species. Thus, incubation time is negatively related to the mean temperature during this period. We assumed that the number of degree-days from egg deposition to larval drift was 150 for walleye (Colby et al. 1979), 225 for quillback (Parker and Franzin 1991), and 220 for white sucker. For white sucker, we assumed that eggs hatched after 120 degree-days (McElman and Balon 1980), but larval drift was delayed by a further 100 degree-days because of the larvae's habit of remaining in the substrate following hatch (Corbett and Powles 1986). Relationships between total annual larval drift and incubation time, water discharge, female stock size, and future year-class strength were examined by regression analysis (GLM procedure, SAS Institute Inc. 1985). All tests of significance were based on the partial (type III) sums of squares. Variables were transformed by $\log_e(x + 1)$ where necessary.

Results

Estimates of total annual larval drift for all fish species in the Valley River during this study ranged from 14 000 in 1984 to >93 million in 1990 (Table 1). Total annual drift was <5 million in all years except 1983 and 1990. Species composition showed considerable variation. White suckers comprised the largest percentage of the drift during our sampling period in most years, followed by walleye (Table 1).

The timing of larval drift differed greatly between years. For walleye, the median date of larval drift ranged from 5 to 27 May, and for white sucker the median date ranged from 12 May to 6 June. The pattern of timing in larval drift, adjusted to the overall mean of median date of drift, is illustrated in Fig. 2. The median date of larval drift differed significantly between walleye, white sucker, and quillback (ANOVA, $F = 50.7$, df = 2, 17, $P < 0.001$). Comparison of means indicated that walleye larvae drifted significantly earlier than white sucker and quillback larvae, but there was no significant difference in median drift date between white sucker and quillback larvae. The median date of walleye drift generally preceded that of the other species by 7 d. Differences between species in the standard deviation of drift date were not statistically significant (ANOVA, $F = 0.65$, df = 2, 17, $P = 0.54$). The CPUE of larval fish in the drift samplers was significantly higher at night than during the day for all three species of larvae captured (Table 2).

Incubation time had no statistically significant effect on the larval drift abundance of any fish species (Table 3). However, abundances of all three species of drifting larvae were significantly affected by spring flows in the Valley River in the 35 d preceding the median date of drift. Minimum discharge appeared to be the best predictor of total annual drift for white sucker and quillback whereas mean discharge was the better predictor for walleye drift abundance (Table 3, Fig. 3). The best discharge predictor variable for each species was able to account for 49–60% of the observed variation in total annual larval drift (Table 3).

Table 2. Comparison of day and night catch per unit effort (CPUE) for various species of fish larvae captured in drift nets set in the Valley River, Manitoba.

Species	Mean CPUE		Test	n	Statistic	P
	Day	Night				
Walleye	629	6271	Wilcoxon's signed rank	25	$T = 29$	<0.005
White sucker	49	1632	Paired-comparisons t	28	$t = 2.05$	0.025
Quillback	46	594	Paired-comparisons t	11	$t = 3.77$	0.0011

Note: Paired samples were collected from approximately 09:00 to 18:00 (day samples), and from 18:00 to 09:00 (night samples) on 35 sampling dates during May from 1988 to 1992. Sample size (n) for a given species represents the number of days on which ≥1 larva was captured in a 24-h period. All tests of significance were one-tailed (H_o: night CPUE ≯ day CPUE vs. H_A: night CPUE > day CPUE). Larval catches were transformed by $\log_e$(CPUE + 1) prior to analysis for all species except walleye. CPUE was calculated as the number of larvae captured in each net per hour.

The addition of incubation time to any of these discharge models did not significantly reduce the residual variance ($F < 0.90$, $P > 0.40$). The independent variable's incubation time and discharge were not significantly correlated for any of the fish species examined (t test, $r < |0.51|$, $P > 0.17$).

When larval abundances were adjusted for the effects of mean discharge, there was a significant, positive effect of mature female biomass on the total annual drift of walleye larvae (Table 4). We also observed a negative relationship between total annual larval drift and mature female biomass of white sucker but this relationship was not statistically significant (Table 4).

The abundance of larval walleye drifting down the Valley River in the spring was reflected in the future year-class strength (Fig. 4). There was a significant, positive relationship between the CPUE of the 3-year-old walleye cohort and the $\log_e$(total annual larval drift) of the same cohort 3 years earlier (ANOVA, $F = 178$, df = 1, 3, $P < 0.001$). No significant relationship was observed between the CPUE of 3-year-old white suckers and total annual larval drift (ANOVA, $F = 0.05$, df = 1, 3, $P = 0.84$). Using the CPUE of 4-year-old rather than 3-year-old fish as an index of year-class strength did not change the outcomes of these tests.

Discussion

Experiments involving controlled releases of known numbers of fish larvae and utilizing drift samplers similar to those used in this study have indicated that estimated larval drift is often low relative to the expected larval drift (Franzin and Harbicht 1992). For this reason, our abundance estimates may be biased low, but patterns in our data probably reflect the actual intraannual and interannual patterns in larval drift, and thus our data are useful indices of larval abundances. Underestimation of larval abundances in the drift could result from an uneven larval distribution across the stream. Several studies have found higher abundances of larvae drifting near the margins of streams rather than near midstream (Brown and Armstrong 1985; Harvey 1991). However, at least one study has noted the opposite trend (Corbett and Powles 1986).

Numerous studies have examined the drift of riverine larval fishes (Elliott 1966; Clifford 1972; Gale and Mohr 1978; Armstrong and Brown 1983; Muth and Schmulbach 1984; Brown and Armstrong 1985; Corbett and Powles 1986; Copp and Cellot 1988; Harvey 1991; Franzin and Harbicht 1992), but none, to our knowledge, has previously examined interannual variation in drift patterns. This study documented large interannual variation in the abundance and composition of drifting larval fishes at a single location. Total annual abundances of drifting larvae during 2 years, 1983 and 1990, were 10–20 times

Fig. 2. Mean distributions of the date of larval fish drift in the Valley River, Manitoba (1982–1992). Annual distributions that differed in timing among years were adjusted to the overall mean of median date of drift for each species or species group.

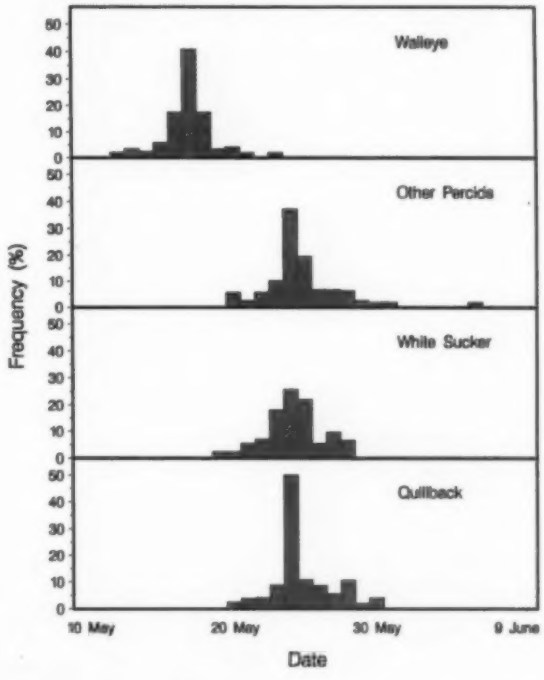


Fig. 3. Scatterplot of $\log_e$(total annual drift) of walleye larvae in the Valley River, Manitoba, from 1982 to 1992 and mean discharge during the 35 d preceding the median date of larval drift.

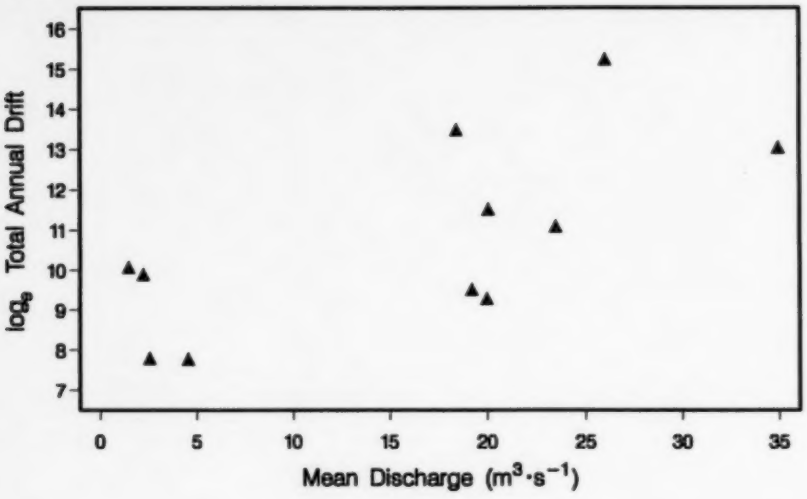


Table 3. Results of simple regressions of $\log_e$(total annual larval drift) of various fish species in the Valley River (1982–1992) against various independent variables, x_i (IT, incubation time (d); Q_{min} and $\bar{Q}$, minimum and mean discharge in the 35 d preceding the median drift date, respectively (m^3·s^{-1})).

Species	x_i	b_i*	F	df	P	R^2
Walleye	IT	0.43	1.16	1, 7	0.32	0.14
	Q_{min}	0.32	1.94	1, 9	0.20	0.18
	$\bar{Q}$	0.15	8.50	1, 9	0.017	0.49
White sucker	IT	−0.73	0.79	1, 7	0.40	0.10
	Q_{min}	0.71	13.8	1, 9	0.0049	0.60
	$\bar{Q}$	0.22	7.3	1, 9	0.024	0.45
Quillback	IT	0.71	1.34	1, 6	0.29	0.18
	Q_{min}	0.79	9.64	1, 7	0.017	0.58
	$\bar{Q}$	0.18	3.64	1, 7	0.098	0.34

*Regression coefficient.

higher than the maximum seen in the remaining 9 years. The composition of the drift showed interannual variation, but in most years white suckers were the dominant species, followed by walleye. Corbett and Powles (1986) also noted larger numbers of drifting white sucker larvae than walleye larvae in southern Ontario streams where the two species spawned sympatrically. Differences in the relative abundances of the various species may be due to a variety of factors including differences in the size of the spawning populations of the two species, habitat availability, or differential egg predation (Corbett and Powles 1986). Despite their abundance in the Valley River, white sucker, walleye, and quillback larvae have rarely been captured in spring ichthyoplankton surveys on Dauphin Lake (Rowes 1994).

Table 4. Results of multiple regressions of $\log_e$(total annual larval drift) of walleye and white sucker in the Valley River (1984–1989) against mean discharge during the 35 d preceding the median date of drift ($\bar{Q}$; m^3·s^{-1}), and $\log_e$(female spawning stock biomass) (SSB; kg/15 gill-net nights).

Species	x_i	b_i*	F	df	P
Walleye	$\bar{Q}$	0.089	10.4	1, 3	0.049
	Female SSB	1.3	14.0	1, 3	0.033
White sucker	$\bar{Q}$	0.31	33.9	1, 3	0.010
	Female SSB	−3.38	7.91	1, 3	0.067

Note: Tests of significance were based on partial (type III) sums of squares. Coefficients of determination (R^2) for the complete models were 0.88 and 0.92 for walleye and white sucker, respectively.
*Regression coefficient.

The timing of larval drift also showed large interannual variation. Median dates of drift varied by 3 weeks over the course of this study. The two latest median dates of drift for both walleye and white sucker were 1983 and 1990, the same 2 years that had the highest total larval drifts. Walleye consistently drifted earlier than other species in the Valley River. Corbett and Powles (1986) noted that white sucker and walleye eggs commenced hatching at the same time but that walleye larvae began drifting almost immediately whereas white sucker larvae remained in the substrate for an additional 11–13 d before drifting. Our estimates of the median and standard deviation of drift date for white sucker were probably biased low because our sampling gear was removed before white sucker larvae finished drifting in several years. Thus, differences in drift timing between white sucker and quillback or walleye may have been greater than our results would suggest.

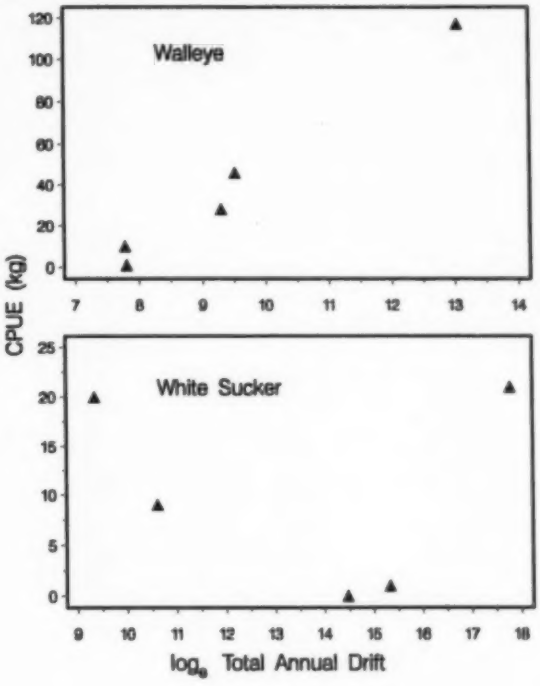
We observed significantly higher CPUEs of larval fishes during the night than during the day. This pattern has been

observed for many species of fish larvae (Elliott 1966; Clifford 1972; Gale and Mohr 1978; Muth and Schmulbach 1984; Brown and Armstrong 1985; Corbett and Powles 1986; Harvey 1991) as well as invertebrates (reviewed by Brittain and Eikeland 1988). Larvae that do drift during the day have been observed to drift deeper in the water column than those drifting at night (Gale and Mohr 1978; Copp and Cellot 1988). Both of these behaviours may reduce predation pressure. An alternative explanation for our results is that fish larvae avoided our sampling gear, passively or actively, during the day. Passive avoidance could result if fish larvae drifted deeper in the water column during the day, thus avoiding our drift bombs, which were always ~20 cm from the surface. Active avoidance seems unlikely because of the high water velocity and turbidity of the water. However, walleye, yellow perch, and even smaller fish larvae appear capable of avoiding active gear such as trawls (Noble 1970; Thayer et al. 1983). Though we feel that the differences between night and day catches observed in this study were much greater than could be explained by active gear avoidance, we suggest that future studies should examine the behaviour of drifting larvae and assess the potential for gear avoidance.

Spring water temperatures and water flows are both considered strong determinants of future year-class strength in percids (reviewed by Koonce et al. 1977). In this study we examined temperature effects on larval abundance by using a variable representing the incubation time from egg deposition to larval drift. We found no statistically significant effect of incubation time on larval drift abundance for any of the fish species examined. We had expected that this relationship would be negative. Busch et al. (1975) hypothesized that shorter incubation times increased the hatching success of lake-spawning walleye eggs because egg mortality was proportional to the length of time the eggs were exposed to the detrimental effects of siltation, dislodging currents, low oxygen concentrations, or predation. Our results did not support this hypothesis. In contrast, the abundance of larval fishes drifting in the Valley River was positively correlated with the river discharge in the 35 d preceding the median date of drift. A higher water discharge during this period may increase the number of adults moving upstream, increase the area of riverbed available for spawning, scour the riverbed of silt and prevent new siltation, reduce upstream movement of egg predators, and ensure sufficient oxygenation for the incubating eggs. Understanding which of these factors, or combination of these factors, is most important in determining the abundance of drifting fish larvae will require further research.

Walleye spawning stock, as estimated by the CPUE of mature females in Dauphin Lake, also had a positive effect on larval walleye drift abundance. Both spring water flows or lake water levels and adult stock size have been found to be important determinants of walleye spawning success in other lakes (Chevalier 1977; Lysack 1986). The theoretical relationship between recruitment and adult stock size is dome shaped. Recruitment increases with adult stock size when stock size is low and decreases with increasing adult stock size when stock size is high (Ricker 1954). During the years 1984–1989, the walleye population of Dauphin Lake was very low relative to historical levels.

Fig. 4. Scatterplots of catch per unit effort (CPUE, number of 3+ fish/15 gill-net nights) for walleye and white sucker in Dauphin Lake from 1985 to 1989 versus total annual drift of walleye and white sucker larvae of the same cohorts in the Valley River 3 years earlier. Note differences in scales of axes between plots.



Thus, a positive relationship between larval walleye abundance and mature female walleye biomass is not surprising. For white sucker, the relationship between larval abundance and mature female biomass may have been negative ($0.05 < P < 0.10$). Such a relationship may indicate that the white sucker population is near carrying capacity. However, information on historical abundances of white suckers in Dauphin Lake is lacking. Unfortunately, these analyses relied on only 6 years of census data (1984–1989) for the adult populations of Dauphin Lake. Thus, our analyses did not include the peak larval drift years of 1983 and 1990.

We found a positive relationship between the year-class strengths of subadult walleye in Dauphin Lake and the abundance of these same cohorts as larvae drifting down the Valley River. High abundances of walleye larvae apparently lead to high abundances of walleye recruiting into the fishery 3 or 4 years later. Similar trends have been observed in some walleye lakes (Carlander and Payne 1977) but not others (Forney 1976). The point at which the strength of a year-class is determined may depend on the adult population and the system in question. One would expect much stronger density-dependent regulation (e.g., older cohorts preying on or competing with juveniles) and much later determination of year-class strengths in populations near carrying capacity. Our results suggest that the year-class

strength of walleye in Dauphin Lake is determined early in the life cycle, whereas the year-class strength of white sucker is probably determined at a later age. Because the walleye population of Dauphin Lake was low during the 1980s relative to historical levels, the negative effect of competition or predation from older year-classes would have been minimal. We predict that the positive relationship between larval abundance and future year-class strength observed in this study will become weaker as the walleye population of Dauphin Lake increases.

The results of this research have several implications for the future management of the Dauphin Lake walleye fishery. First, the strength of walleye year-classes depends upon the abundance of walleye larvae entering the lake in the spring. Monitoring the abundance of these early life stages could be an important management tool for predicting recruitment and adjusting harvest levels accordingly. Second, the abundance of naturally produced walleye larvae depends on physical conditions in the spawning rivers and the abundance of mature females. Thus, management of the Dauphin Lake walleye fishery could take several approaches to increase the walleye population. Natural production of larvae could be enhanced by regulating water flows, or increasing the escapement of adult females from the fishery. Alternatively, the lake could be stocked with hatchery-reared larvae. Regulating water flows in the spawning rivers would be a difficult task. The volume of spring flow is largely a function of winter and spring precipitation, whereas the intensity and duration of spring flow depend both on precipitation and the physical characteristics of the drainage basin. Extensive land clearing in the Dauphin Lake drainage basin during the past 80 years has resulted in higher peak flows and shorter durations of elevated flows during the spring spawning season (Gaboury 1985). Halting land clearing, particularly in the riparian zones of spawning rivers, may prevent further alterations to flow regimes. Stocking larvae into lakes with naturally reproducing walleye populations has generally had little effect (reviewed by Laarman 1978; Ellison and Franzin 1992). An introduction of 17 million walleye larvae into Dauphin Lake in the spring of 1985 contributed only 2.9% to the 1985 year-class 3 years later (Mathias et al. 1992). Over the 11 years of monitoring larval drift in the Valley River, 1985 had the fourth lowest total annual drift of walleye larvae. The fact that a relatively weak cohort of naturally produced walleye larvae dominated a rather large infusion of hatchery-produced larvae 3 years later suggests that larval stocking may be ineffective in this system.

Acknowledgements

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Influence of fluoride on aluminum toxicity to Atlantic salmon (*Salmo salar*)

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Abstract: Atlantic salmon (*Salmo salar*) alevins were exposed to various aluminum (0–4700 μg/L) and four fluoride (0–500 μg/L) concentrations at two pH values (5.5 and 6.5) for 4- and 30-d periods. In the 4-d tests, aluminum with fluoride was less toxic at pH 6.5 than at pH 5.5, whereas without fluoride, pH had no effect. In the 30-d test, mortality in all treatments was 17–21% at pH 5.5, but only 3–7% at pH 6.5. Fish length and weight after 30 d were reduced in all fluoride–aluminum treatments at pH 5.5, but only in the 200-μg/L aluminum without fluoride treatment at pH 6.5. At pH 5.5 and 6.5 without aluminum, histomorphological examinations revealed no abnormalities in gill tissue. However, in aluminum exposure with no fluoride, gill filaments and secondary lamellae were swollen and thickened. Addition of fluoride at pH 6.5 alleviated some gill damage. At pH 5.5 and 200 μg/L aluminum, addition of 100 μg/L fluoride reduced swelling of gill lamellae, but 200 μg/L fluoride did not reduce swelling. Low fluoride concentrations (<100 μg/L) may reduce gill morphological damage in fish exposed to aluminum in acidic waters, whereas high fluoride concentrations (>100 μg/L) may not reduce aluminum-induced effects.

Résumé : Des alevins de saumon atlantique (*Salmo salar*) ont été exposés à diverses concentrations d'aluminium (0–4700 μg/L) et à quatre concentrations de fluorure (0–500 μg/L), avec 2 pH différents (5,5 et 6,5), pendant des périodes de 4 et 30 jours. Dans les tests de 4 jours, l'aluminium était, en présence de fluorure, moins toxique à 6,5 qu'à 5,5 de pH, mais, en l'absence de fluorure, le pH n'avait aucun effet. Dans le test de 30 jours, la mortalité était, pour tous les traitements, de 17–21% à 5,5 de pH, mais seulement de 3–7% à 6,5 de pH. La longueur et le poids des poissons après 30 jours étaient réduits dans tous les traitements fluorure–aluminium à 5,5 de pH, mais seulement dans le traitement à 200 μg/L d'aluminium sans fluorure à 6,5 de pH. À 5,5 et 6,5 de pH, sans aluminium, les examens histomorphologiques n'ont révélé aucune anormalité dans les tissus des branchies. Par contre, dans l'exposition à l'aluminium en l'absence de fluorure, les filaments branchiaux et les lamelles secondaires étaient enflés et épaissis. L'ajout de fluorure à 6,5 de pH atténuait une partie des dommages aux branchies. À 5,5 de pH et en présence de 200 μg/L d'aluminium, l'ajout de 100 μg/L de fluorure réduisait l'enflure des lamelles, mais 200 μg/L de fluorure n'avaient pas cet effet. De faibles concentrations de fluorure (<100 μg/L) peuvent réduire les dommages morphologiques aux branchies chez les poissons exposés à l'aluminium dans des eaux acides, tandis que de fortes concentrations de fluorure (>100 μg/L) ne réduisent pas forcément les effets de l'aluminium.

[Traduit par la Rédaction]

Introduction

In water, aluminum complexes with naturally occurring organic ligands, and hydroxide, fluoride, and sulfate ions. Organic aluminum is usually the predominant fraction, and fluoride complexes are the dominant inorganic species (Driscoll et al. 1980; Driscoll 1985). Complexation of aluminum by organic acids can reduce the toxic effects of aluminum on fish growth and survival (Baker and Schofield 1980; Driscoll et al. 1980; Peterson et al. 1989). Aluminum–fluoride complexes have been reported to be somewhat less toxic to fish than hydroxide complexes, although results are highly variable (Baker and Schofield 1980; Driscoll et al. 1980; Fivelstad and Leivestad 1984; Lydersen et al. 1990; Wilkinson et al. 1990). Further, aluminum–fluoride complexes are not altered by changes in water temperature, whereas aluminum hydroxides increase with increasing water temperature (Poleo et al. 1991).

Acidic precipitation has caused acidification of numerous rivers throughout the world, including salmon rivers in

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southern Norway (Overrein et al. 1981) and southwestern Nova Scotia (Watt et al. 1983). As a result, Atlantic salmon (*Salmo salar*) populations in those rivers severely affected have been either reduced or eliminated. Because of similarities in climatic and geologic conditions between these areas and the northeastern United States, concern has also been raised about Atlantic salmon here (Haines 1992). In the northeastern United States both present and historical habitats of Atlantic salmon receive precipitation that is highly acidic; the mean annual volume-weighted pH is 4.3–4.6 (National Atmospheric Deposition Program 1992).

Atlantic salmon nursery streams in the northeastern United States are highly variable in fluoride content. Haines et al. (1990) found that total fluoride concentrations varied between 0 and 100 μg/L in one drainage basin in Maine but reached concentrations as high as 475 μg/L in an adjacent drainage. Fluoride concentrations were highest, and pH lowest, during high flow periods in these streams. Therefore, aluminum–fluoride complexes could be a very important factor in the formation of inorganic complexes and consequently in the toxicity of aluminum to Atlantic salmon in some streams.

This study was undertaken to assess the influence of fluoride complexation on the toxicity of aluminum to young Atlantic salmon in low alkalinity, low pH waters. In acute and chronic toxicity tests, survival, growth, and gill histopathology were measured.

Materials and methods

Atlantic salmon alevins were obtained from the Craig Brook National Fish Hatchery in East Orono, Maine. Fish were briefly cultured in Fiberglass troughs (41 × 213 × 11 cm) in well water at 17 ± 2°C (mean ± SD) (500 mg/L hardness as $CaCO_3$, 281 mg/L alkalinity as $CaCO_3$, and pH 7.2). Fish were fed a standard commercial salmon diet (Bioproducts, Inc.).[1]

Acute exposure

Tests were conducted with aluminum chloride (CAS No. 7446-70-0; 20.24% aluminum; Aldrich Chemical Co., Milwaukee, Wis.) and sodium fluoride (CAS No. 7681-49-4; 44.8% fluoride; Aldrich Chemical Co.). Stock solutions were prepared in deionized water, in acid-washed 100-mL volumetric flasks on the day of use. Test solutions were prepared by pipetting appropriate aliquots of stock solution into test vessels. Concentrations were expressed as total inorganic element added, as determined from the certificate of analysis for each compound. For mixture tests, stock solutions for each component were prepared separately and the chemicals added sequentially.

Toxicity tests were conducted in reconstituted dilution water that simulated typical soft, low-color waters in Maine. The water was prepared by adding $CaSO_4$, $MgSO_4$, NaCl, $NaHCO_3$, and KNO_3 to deionized water in a 5700-L polyethylene tank equipped with a recirculation pump to mix and aerate the water (Hamilton et al. 1989). The dilution water used in acute toxicity tests had the following characteristics: 6.6–7.0 mg/L hardness as $CaCO_3$, 1.66–1.69 mg/L alkalinity as $CaCO_3$ (33–34 μequiv./L), and 33 μmhos/cm (1 mho = 1 S) (25°C) conductivity. Water quality characteristics were measured for each tank of dilution water as follows: calcium, sulfate, total hardness, and total alkalinity were determined according to the U.S. Environmental Protection Agency (EPA) (1979); magnesium concentrations were calculated from calcium and hardness values; chloride was measured with a Buchler chloridometer; specific conductance (corrected to 25°C) was measured with a YSI model 31 conductivity bridge with a cell constant of $K = 1.0$; and pH was determined with an Orion model 901 Ionalyzer with a Ross combination electrode.

Static 4-d toxicity tests were conducted according to procedures recommended by the American Society for Testing and Materials (1989). Static exposures were conducted in 3.8-L glass jars containing 3 L of test solution maintained at 12 ± 1°C in temperature-controlled water baths. One series of four tests was conducted at pH 5.5 and a second series of four tests at pH 6.5. In each test series, four concentrations of fluoride were tested (0, 50, 250, and 500 μg/L). In each test, groups of 10 sac fry (≈0.2 g) were exposed to a geometric series of six to eight aluminum concentrations (28–4700 μg/L) with a 60% dilution factor between treatments and a control. The pH of the dilution water was measured and adjusted daily in all test vessels and adjusted daily using sulfuric acid. Dissolved oxygen was measured at 0, 48, and 96 h of exposure in the control, low, middle, and high treatment test vessels using a YSI model 58 dissolved-oxygen meter.

Fish were acclimated to the dilution water and temperature for 2 d under static renewal conditions and held in the dilution water for 2 d prior to testing. The fish were not fed during either acclimation or testing. At the start of each test, fish were randomly distributed to the test vessels within 30 min after addition of the toxicant. Fish were carefully netted from the holding tank and distributed in groups of two to each test vessel. Mortality and abnormal behavioral responses were recorded, and all dead fish were removed at 24-h intervals.

The moving average angle method or probit method (Peltier and Weber 1985) was used to calculate the LC_{50} values and 95% confidence intervals, depending on the number of mortalities in a test. All LC_{50} values are expressed as nominal concentrations of the aluminum and fluoride tested. The standard error of the difference, as described by Sprague and Fogels (1977) and Zar (1974), was used to determine significant differences ($p = 0.05$) between LC_{50} values.

Chronic exposure

A 30-d test was conducted with sac fry exposed to aluminum and fluoride in two pH treatments. For each pH treatment, fish were exposed to nominal concentrations of 0 μg/L aluminum and 0 μg/L fluoride (control), 200 μg/L aluminum and 0 μg/L fluoride, 200 μg/L aluminum and 100 μg/L fluoride, and 200 μg/L aluminum and 200 μg/L fluoride. The test was conducted in a modified flow-through proportional diluter system (Mount and Brungs 1967). Flow-splitting chambers (Benoit and Puglisi 1973) were used to thoroughly mix and divide each concentration for

[1] References to trade names, commercial products, or manufacturers do not imply or constitute government endorsement or recommendation for use.

Table 1. Water quality in aquaria where Atlantic salmon were exposed to aluminum and fluoride at two pH levels for 30 d.

Variable	pH 5.5	pH 6.5
pH	5.51±0.07	6.41±0.05
Alkalinity (μequiv./L)	4.7±1.5	46.1±4.6
Hardness (mg/L as $CaCO_3$)	8.9±0.4	8.6±0.3
Conductivity (μS/cm)	52.9±3.8	49.5±3.2
Calcium (mg/L)	3.0±0.1	2.8±0.1
Magnesium (mg/L)	0.3±0	0.4±0.1
Chloride (mg/L)	5.2±1.0	5.4±0.8
Sulfate (mg/L)	8.9±0.2	6.5±0.1
Ammonia (mg/L)	0.2±0	0.2±0

Note: Values are given as the mean ± standard error (n = 16).

Table 2. Acute toxicity (96-h LC_{50} with 95% confidence interval given in parentheses) for Atlantic salmon exposed to aluminum and selected amounts of fluoride at two pH levels.

pH	Fluoride (μg/L)	LC_{50} for aluminum (μg/L)
5.5	0	584*a* (444–676)
	50	680*a* (624–737)
	250	478*b* (444–525)
	500	478*b* (444–525)
6.5	0	599*x* (445–772)
	50	1134*y** (843–1444)
	250	716*x** (574–875)
	500	734*x** (588–903)

Note: Values followed by the same letter (within the same pH) are not significantly different (p = 0.05). Values with an asterisk within the same fluoride treatment are signficantly different (p = 0.05).

delivery through glass tubes to duplicate 46-L exposure aquaria. Each aquarium received 1 L of water every 15 min, resulting in a 90% replacement time of about 27 h on the basis of Sprague's monograph (Sprague 1969). Reconstituted dilution water was the same as used for the 4-d exposures. The photoperiod was controlled by a timed artificial lighting system (Drummond and Dawson 1970) to simulate conditions in Maine for April. Oxygen concentrations in exposure water were supplemented by passing compressed air from an oilless air compressor through airstones placed in each chamber of exposure aquaria. Aeration ensured adequate dissolved oxygen concentrations, as well as homogeneity of pH and aluminium and fluoride levels throughout each chamber. Aluminum, fluoride, and sulfuric acid stock solutions were prepared in deionized water and delivered to the diluter by an automatic pipetting system. Test temperature was maintained at 12 ± 2°C by placing eight exposure aquaria in each of two temperature-controlled water baths that were connected to allow continuous, pump-aided water circulation.

Each of 16 aquaria was stocked with 100 alevins: 60 in a large 23-L chamber from which histology samples were collected, and 20 each in two 11.4-L chambers from which fish growth measurements were made. Eight aquaria separated in two blocks of four, replicates A and B, were held in separate water baths. Aquaria in one water bath were maintained at pH 5.5 and those in the second water bath at pH 6.5. Fish were allowed to gradually acclimate to the dilution water and test temperature for 3 d, with the acid treatment introduced on the second day and the aluminum and fluoride treatments introduced on the fourth day. The test fish were fed ad libitum with Biodiet, supplemented twice daily with live brine shrimp nauplii. Survival was recorded daily, and dead fish were removed. At the end of the study, growth effects were determined by measuring total length and weight of fish in the two 20-fish subgroups held in 11.4-L chambers within each aquarium. Length was measured to the nearest 0.1 mm and weight to the nearest 0.01 g.

After 30 d of exposure, five fish from the 23-L chamber of each exposure aquarium were randomly sampled for histological examination of the gills. Their heads were severed just posterior to the opercular openings and immediately fixed in an ice-cold mixture of 1% gluteraldehyde and 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4) containing 5% sucrose (Jagoe et al. 1993). Tissues were refrigerated until examined.

Water samples were collected for analysis of aluminum, fluoride, and other ions at 30 d of exposure. One set of 250-mL samples was immediately frozen and stored at −20°C until analysis of anion concentrations (chloride, fluoride, nitrate, and sulfate). A second set of 100-mL samples was preserved with 1 mL of concentrated ultrapure nitric acid, but not frozen, and analyzed for total aluminum and cation concentrations (ammonia, calcium, potassium, magnesium, sodium, and silica). A third set of 250-mL samples was filtered through an ion exchange column (Dowex 50, 20–50 mesh, sodium form); the first 100 mL was discarded, then 100 mL was collected and acidified to pH 0–1 with ultrapure nitric acid for measurement of organic aluminum. Exchangeable (also termed labile monomeric or ionic) aluminum was calculated as the difference between total aluminum and organic aluminum. Calcium, magnesium, sodium, and potassium were determined by flame atomic absorption spectrophotometry; aluminum by graphite furnace atomic absorption spectrophotometry; sulfate, nitrate, chloride, and fluoride by ion chromatography; and silica by automated colorimetry (U.S. Environmental Protection Agency 1987). The pH was measured in all aquaria daily, and the same water quality characteristics as for the acute toxicity tests were measured in one replicate of eight aquaria weekly (Table 1).

Histopathology

Gill morphology was assessed by dissecting the second and third gill arches from the right side of the preserved heads, postfixing in 1% OsO_4 for 2 h, dehydrating in a graded ethanol series, then critical-point drying under CO_2 (Jagoe et al. 1993). Dried arches were mounted on specimen stubs, sputter coated with a gold–palladium mixture,

Table 3. Percent mortality of Atlantic salmon exposed for 15 and 30 d to aluminum and fluoride in water with low alkalinity and calcium.

Aluminum (μg/L)	Fluoride (μg/L)	Days of exposure 15	30
		pH 5.5	
0	0	12.0±8.0*a*	21.5±8.5*b*
200	0	0±0*a*	19.5±7.5b
200	100	6.0±6.0*a*	20.0±11.0*b*
200	200	1.5±0.5*a*	17.5±0.5*b*
		pH 6.5	
0	0	0±0*x*	3.5±1.5*x*
200	0	2.0±1.0*y*	4.5±0.5*xy*
200	100	0.5±0.5*xy*	2.5±0.5*x*
200	200	0±0*x*	7.5±0.5*y*

Note: Values are given as the mean ± standard error. Aluminum–fluoride treatments (within the same pH and day) followed by the same letter are not significantly different (ANOVA, $p < 0.10$). There was a significant ($p < 0.05$) difference between pH treatments at day 30, but not day 15. There was a significant difference between day 15 and 30 for pH 5.5 treatments, and a significant interaction between pH and the aluminum–fluoride group for pH 6.5.

and examined with an AMR 100A scanning electron microscope operated at 15 kV.

Aluminum speciation

Aluminum speciation was calculated from measured total aluminum and ion content data using the thermodynamic equilibrium computer program ALCHEMI (Schecher and Driscoll 1987).

Statistics

Aquaria were considered the experimental unit. A repeated measures design was used to determine the effects of mortality across time. Effects of pH and toxicants on percent survival (arcsine-transformed values) and growth were evaluated by analysis of variance using the Statistical Analysis System (SAS Institute Inc. 1985). Treatment means were compared with a least significant difference test for all main-effect means: pH and group, where group was each aluminum–fluoride combination.

Results

Acute exposure

In the absence of fluoride, pH alone had no effect on aluminum toxicity to Atlantic salmon (Table 2). However, in the presence of fluoride, aluminum was less toxic at pH 6.5 than at pH 5.5. At pH 5.5, aluminum was more toxic in the 250 and 500 μg/L fluoride treatments than in the 50 μg/L fluoride treatment. At pH 6.5, aluminum was less toxic in the presence of 50 μg/L fluoride than in the 0, 250, and 500 μg/L fluoride treatments. Thus, at pH 5.5, the high fluoride concentration (250 and 500 μg/L) apparently increased the toxicity of aluminum, whereas at pH 6.5, the low fluoride concentration (50 μg/L) reduced the toxicity of aluminum to young Atlantic salmon. Overall, aluminum toxicity was reduced in the presence of fluoride at pH 6.5 compared with pH 5.5.

Table 4. Total length, weight, and condition in Atlantic salmon exposed for 30 d to aluminum and fluoride in water with low alkalinity and calcium.

Aluminum (μg/L)	Fluoride (μg/L)	Total length (mm)	Weight (g)	Condition
		pH 5.5		
0	0	35.2±0.1*a*	0.34±0*a*	0.77±0.01*a*
200	0	32.1±0.2*b*	0.23±0.01*b*	0.71±0.01*b*
200	100	33.1±0.8*ab*	0.28±0.02*b*	0.76±0.01*a*
200	200	32.0±0.2*b*	0.25±0.01*b*	0.74±0.01*a*
		pH 6.5		
0	0	34.7±0.6*xy*	0.34±0.02*x*	0.79±0.01*x*
200	0	31.6±1.1*y*	0.23±0.03*y*	0.73±0.03*x*
200	100	35.8±0.6*x*	0.36±0.02*x*	0.76±0*x*
200	200	36.4±0.6*x*	0.39±0.02*x*	0.79±0.01*x*

Note: Values are given as the mean ± standard error. Aluminum–fluoride treatments (within the same pH) followed by the same letter are not significantly different (ANOVA test, $p < 0.05$). Condition = weight/(length)^3 × 100 000.

Chronic exposure

Mortality of Atlantic salmon alevins was significantly higher at 30 d of exposure in pH 5.5 treatments than in pH 6.5 treatments, but there was no difference between pH treatments at 15 d. Within pH 5.5 treatments, mortality was higher after 30 d of exposure than after 15 d (Table 3). In the pH 6.5 treatments, a significant interaction between the aluminum–fluoride treatments and time precluded determining if mortality was statistically different between day 15 and 30. For pH 5.5 treatments, there were no significant differences in mortality between aluminum–fluoride treatments at either exposure duration. At pH 6.5, the magnitude of the differences in mortality between aluminum–fluoride groups, although significant, was not large compared with the control.

When compared with fish from the control treatment at pH 5.5 (no added aluminum or fluoride), Atlantic salmon from all aluminum–fluoride treatments at pH 5.5 weighed less, but there were no differences in fish length between aluminum–fluoride treatments (Table 4). At pH 6.5, there were significantly lower fish lengths and weights in the 200 μg/L aluminum and 0 μg/L fluoride treatment than in the control treatment. In contrast to the pH 5.5 results, the presence of 100 or 200 μg/L fluoride at pH 6.5 apparently ameliorated adverse effects on fish weight. This observation parallels the results in acute toxicity tests where the presence of fluoride at pH 6.5 reduced aluminum toxicity. Regardless of pH treatment, the predominant reduction in fish length and weight was in the aluminum exposures with no added fluoride.

Fig. 1. Scanning electron micrographs of gills from fish exposed to various concentrations of aluminum and fluoride at pH 6.5. (A) Al, 0 μg/L; F, 0 μg/L. Scale bar = 100 μm. (B) Al, 0 μg/L; F, 0 μg/L. Scale bar = 10 μm. (C) Al, 200 μg/L; F, 0 μg/L. Scale bar = 100 μm. (Note rounded tips, thicker filaments, and secondary lamellae in C compared with A.) (D) Al, 200 μg/L; F, 0 μg/L. Scale bar = 10 μm. (E) Al, 200 μg/L; F, 100 μg/L. Scale bar = 100 μm. Arrow, lesion. (F) Al, 200 μg/L; F, 100 μg/L. Scale bar = 10 μm. Arrow, lesion. (G) Al, 200 μg/L; F, 200 μg/L. Scale bar = 100 μm. Arrow, lesion. (H) Al, 200 μg/L; F, 200 μg/L. Scale bar = 10 μm. Arrow, lesion.

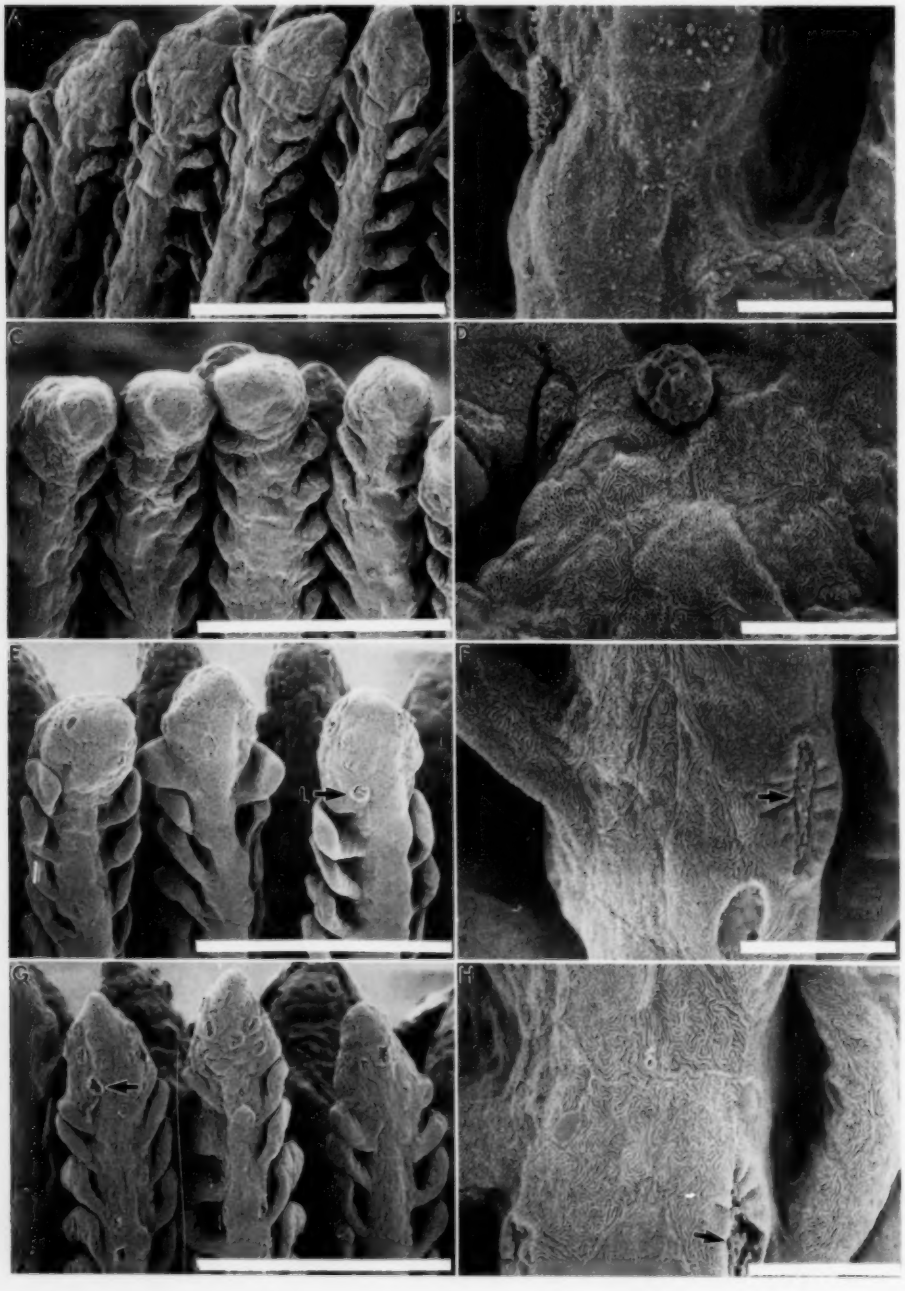


Table 5. Chronic exposure water pH, amount of aluminum and fluoride added, and predicted aluminum species present.

Aluminum (μg/L)		Fluoride (μg/L)		Aluminum present in various forms (μg/L, calculated)			
Nominal	Measured	Nominal	Measured	Ionic	H-bound	F-bound	Total
			pH 5.5				
0	1.0±1.4	0	0	0	1	0	1
200	79±4.2	0	0	47	30	0	77
200	77±1.4	100	109±0	0	39	72	111
200	106±4.2	200	257±13.4	0	46	103	149
			pH 6.5				
0	2.0±0.5	0	0	0	1	0	1
200	222±40.3	0	0	3	118	0	121
200	84±8.5	100	114±0	0	75	56	131
200	88±2.1	200	238±13.4	0	71	74	145

Note: Aluminum and fluoride values are given as the mean ± standard deviation. Ionic, Al_3; H-bound, hydroxide forms combined (AlOH, $Al(OH)_2$, $Al(OH)_4$); F-bound, fluoride forms combined (AlF, AlF_2, AlF_3, AlOHF, $AlOHF_2$, $Al(OH)_3F$).

Histopathology

On histological examination, the gills of fish exposed to the pH 6.5 control (no added aluminum) appeared normal (Figs. 1A and 1B). Secondary lamellae were thin and distinct, epithelial cells were not swollen, and surface microridges were distinct. Addition of aluminum in the absence of fluoride resulted in marked thickening of gill filament and secondary lamellae (Figs. 1C and 1D). Filament tips were blunt in appearance, the filament shaft seemed wider than in the control fish, and secondary lamellae were short and thick, with some fusion of adjacent lamellae. Epithelial cells were notably swollen, but microridges remained distinct. The addition of 100 μg/L fluoride alleviated the thickening of filaments and secondary lamellae, eliminated fusion of secondary lamellae, and reduced swelling of epithelial cells (Figs. 1E and 1F). The condition of gills following addition of 200 μg/L fluoride was similar to that for the 100 μg/L exposure, with some swelling and fusion of secondary lamellae apparent (Figs. 1G and 1H). However, what seemed to be open lesions were apparent on the gill filaments at both fluoride concentrations (Figs. 1E–1H). Such lesions did not occur in any treatment without fluoride.

At pH 5.5 in the absence of aluminum, the appearance of gills was similar to that observed at pH 6.5, i.e., normal (Figs. 2A and 2B). The addition of aluminum resulted in massive swelling of gill epithelial cells (Figs. 2C and 2D). The gill filaments appeared wrinkled and secondary lamellae were greatly reduced, with fusion of adjacent lamellae. The addition of fluoride reduced, but did not eliminate, this swelling (Figs. 2E–2H). Gills from the 200 μg/L fluoride treatment seemed more swollen than those from the 100 μg/L treatment and resembled gills from the pH 6.5 with aluminum treatment (Figs. 2E–2H). The lesions seen when fluoride was added to the pH 6.5 treatments were absent at pH 5.5, but at the 200 μg/L fluoride treatment, pores, probably chloride cell apical crypts, were evident in the gill filament (Fig. 2H).

Aluminum speciation

The measured concentrations of total aluminum and fluoride are given in Table 5. The reason for the poor correspondence between nominal and measured concentrations of aluminum is unknown. The summed species, calculated with the ALCHEMI computer program, are shown individually in Figs. 3 and 4 for pH 6.5 and 5.5, respectively. In the pH 6.5 chronic exposure treatments with no added fluoride, most aluminum was found as $Al(OH)_2$ species (Fig. 3). With the addition of fluoride (100 and 200 μg/L) the proportion of aluminum in the $Al(OH)_2$ form progressively declined, and the fluoride-bound species increased. With both fluoride additions, most aluminum was in the AlOHF form.

At pH 5.5 in the absence of fluoride, the aluminum species distribution varied between the treatments with 0 and 200 μg/L aluminum added (Fig. 4). When aluminum was added, the majority was found in the Al^{3+} form, rather than in the $Al(OH)_2$ form as found at pH 6.5. The addition of fluoride essentially eliminated the Al^{3+} form and greatly reduced the $Al(OH)_2$ form, replacing them with the fluoride-bound forms AlOHF and AlF_2.

Discussion

Acute exposure

The amount of fluoride added in our tests was substantially below the 8-d LC_{50} of 64 mg/L for rainbow trout (*Oncorhynchus mykiss*) and 97 mg/L for brown trout (*Salmo trutta*) reported by Camargo and Tarazona (1991). Consequently, fluoride by itself probably did not contribute to toxicity in our tests.

Our 96-h LC_{50} for Atlantic salmon exposed to aluminum at pH 6.5 (no added fluoride) is six times lower than the

Fig. 2. Scanning electron micrographs of gills from fish exposed to various concentrations of aluminum and fluoride at pH 5.5. (A) Al, 0 μg/L; F, 0 μg/L. Scale bar = 100 μm. (Note fusion of secondary lamellae near tip (arrow) compared with Fig. 1A.) (B) Al, 0 μg/L; F, 0 μg/L. Scale bar = 10 μm. (C) Al, 200 μg/L; F, 0 μg/L. Scale bar = 100 μm. (D) Al, 200 μg/L; F, 0 μg/L. Scale bar = 10 μm. (E) Al, 200 μg/L; F, 100 μg/L. Scale bar = 100 μm. (F) Al, 200 μg/L; F, 100 μg/L. Scale bar = 10 μm. (G) Al, 200 μg/L; F, 200 μg/L. Scale bar = 100 μm. Arrow, chloride cell apical crypt. (H) Al, 200 μg/L; F, 200 μg/L. Scale bar = 10 μm. Arrow, chloride cell apical crypt.

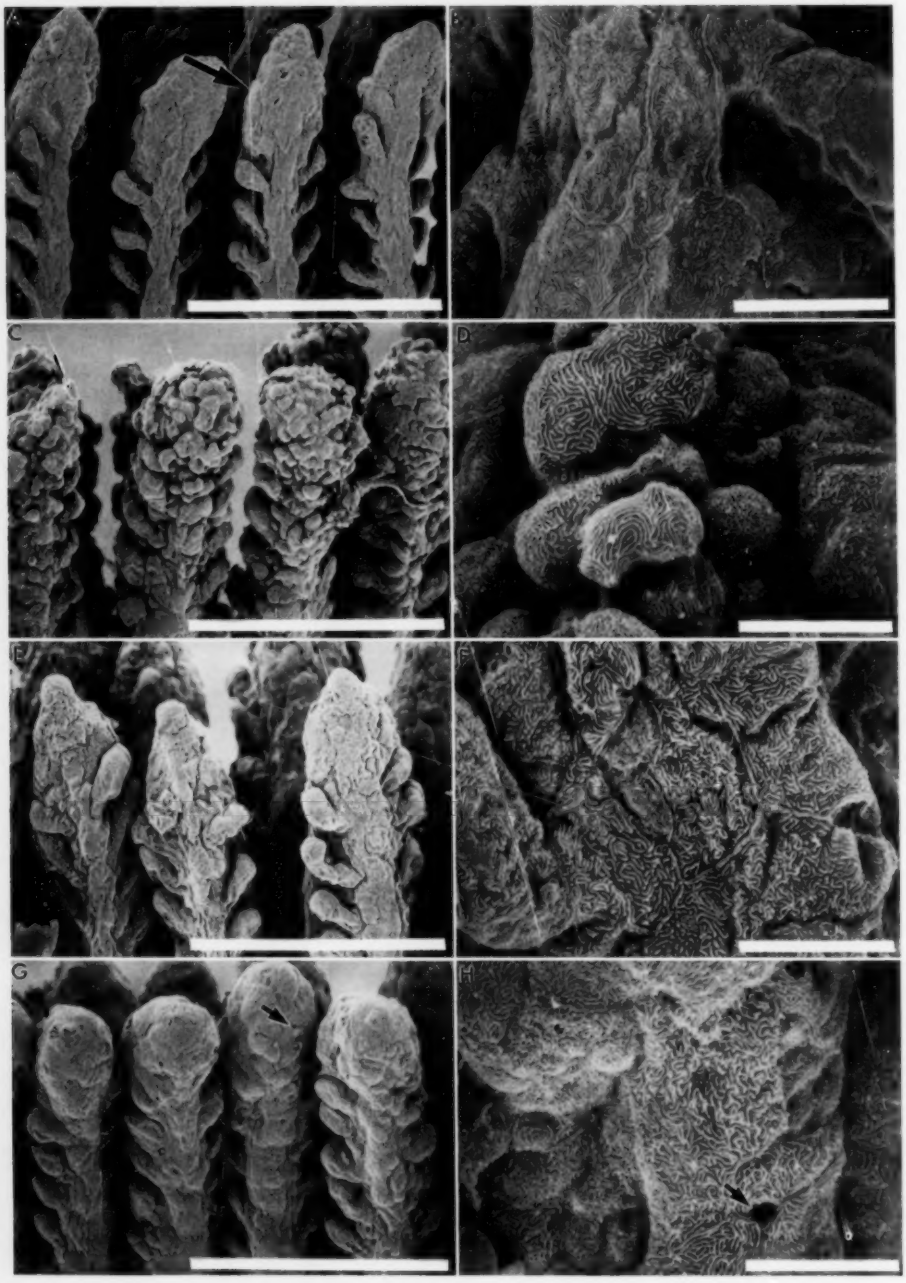
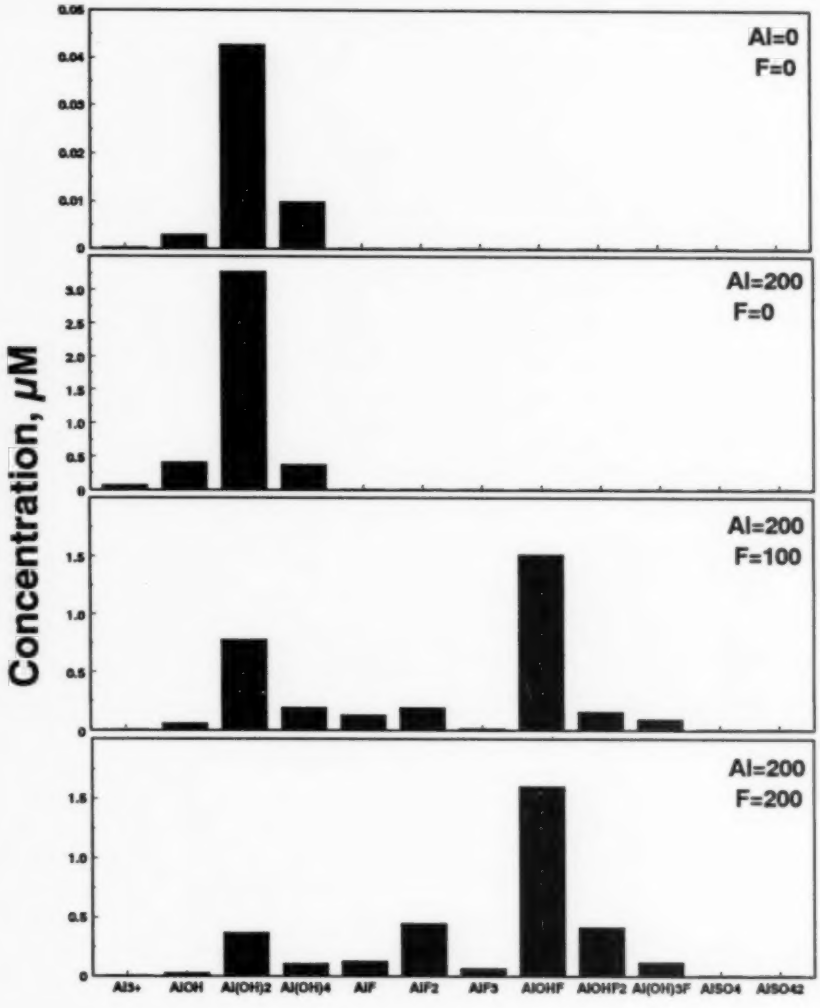


Fig. 3. Calculated concentrations of aluminum species for various combinations of aluminum and fluoride at pH 6.5.

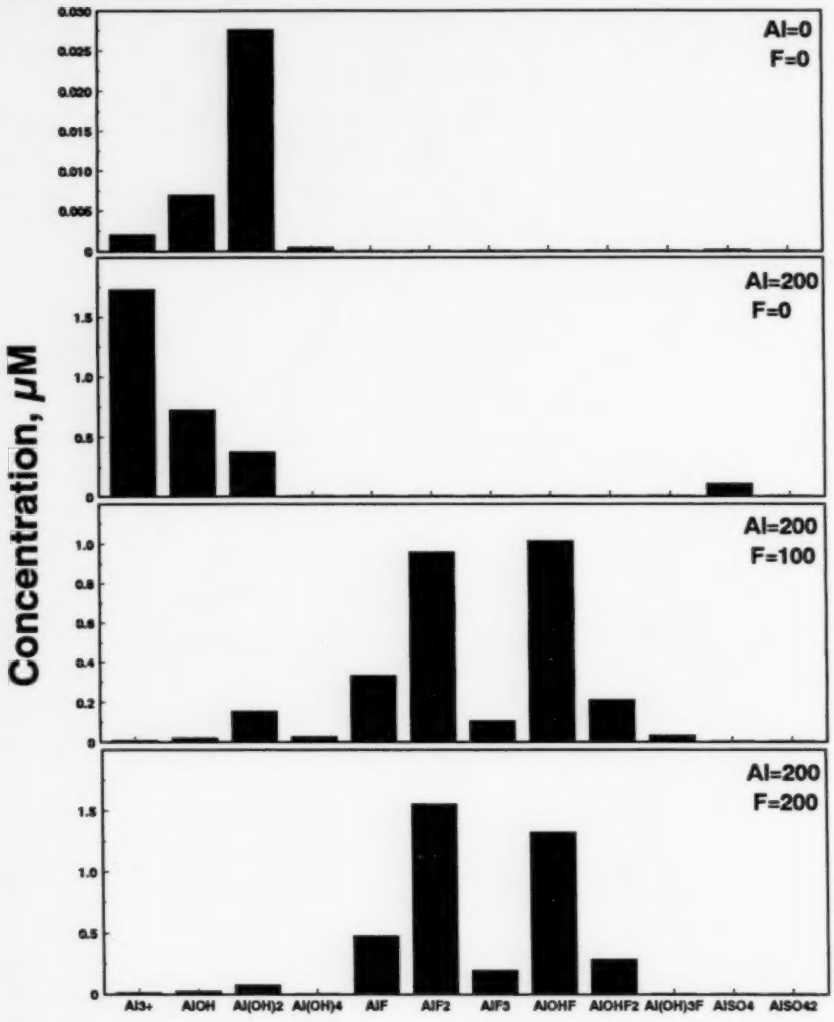


lowest toxic value reported for Salmonidae in a recent review (Gostomski 1990). The lowest value reported was for 14-month-old brook trout (*Salvelinus fontinalis*) exposed to aluminum sulfate at pH 6.5 (Decker and Menendez 1974). Their 96-h LC_{50} was 3600 μg/L aluminum. However, our value for pH 5.5 and no added fluoride (584 μg/L) is higher than the 96-h LC_{50} of 130 μg/L aluminum for smallmouth bass (*Micropterus dolomieu*) exposed at pH 5.1 in soft water (12 mg/L hardness, 3 mg/L calcium; Kane and Rabeni 1987). Laboratory aluminum toxicity data vary widely because of differences in the saturation of the aluminum solutions used (Spry and Wiener 1991). All solutions in our tests were undersaturated for $Al(OH)_3$, as calculated by the ALCHEMI computer program.

Our results indicate that at pH 5.5, low fluoride addition (50 μg/L) did not influence the acute toxicity of aluminum, but at high fluoride (250 or 500 μg/L) aluminum toxicity was increased. At pH 6.5, low fluoride addition (50 μg/L) reduced aluminum toxicity, whereas at high fluoride concentrations (250 or 500 μg/L), aluminum toxicity was not significantly influenced. These results are somewhat similar to the findings of Wilkinson et al. (1990), who reported that fluoride complexation with aluminum at pH 4.5–4.9 altered the toxicity of aluminum to Atlantic salmon. In a 7-d test (their experiment A) with constant monomeric aluminum (234 μg/L) and variable fluoride (13–194 μg/L), they found that mortality was 100% in 2 d when 25 μg/L fluoride was added, but 0% after 7 d when 194 μg/L fluoride was added. However, in two other 7-d studies (their experiments B and C), they showed that when the sum of the Al^{3+} and $Al(OH)_n$ concentrations was held constant, the mortality of Atlantic salmon increased with increasing concentrations of aluminum–fluoride complexes.

Baker and Schofield (1980) and Driscoll et al. (1980)

Fig. 4. Calculated concentrations of aluminum species for various combinations of aluminum and fluoride at pH 5.5.



reported that fluoride reduced the toxicity of aluminum in 14-d tests with brook trout fry in low alkalinity water at pH 4.4 and 5.2, similar to the findings of Wilkinson et al. (1990, experiment A). Lydersen et al. (1990) also reported intoxication of Atlantic salmon alevins when exposed to aluminum–fluoride complexes in two low pH (4.8–5.2) lakes in Norway. However, they found that the fluoride complexes of aluminum were less toxic than other aluminum forms (i.e., free aluminum or hydroxide complexed). Our results are supported by those of Wilkinson et al. (1990, experiments B and C). That is, fluoride can appreciably reduce aluminum toxicity in some situations, but not in others, and in some situations toxicity may even be increased by fluoride addition.

Chronic exposure

The chronic exposure conditions in our study were intended to be sublethal to young Atlantic salmon. Therefore, sublethal indicators of stress such as growth or gill morphology were employed as measures of effects. Others have reported that exposure of early life stages of salmonids to low aluminum concentrations reduces growth without causing significant mortality (Siddens et al. 1986; Gunn and Noakes 1987; McKee at al. 1989), similar to our results. Juvenile brook trout exposed to 200 μg/L aluminum at pH 4.9 had reduced growth, but no mortality during a 24-d test by Siddens et al. (1986). After 5-d exposure to 100–200 μg/L aluminum at pH 5.0 in 5 mg/L hardness water then recovery for 21 d, alevins of lake trout (*Salvelinus*

namaycush) had reduced length and calcification of skeletal elements but no mortality (Gunn and Noakes 1987). Likewise, McKee et al. (1989) reported that growth, as measured by RNA and DNA content, of larval Atlantic salmon exposed to aluminum in pH 5.5 in soft water was a more sensitive indicator of toxicant effects than mortality. Our results are similar to these in that we found little or no difference in mortality within pH treatments (Table 3), yet there were significant reductions in growth in length and weight (Table 4). In contrast, Reader et al. (1988) reported that 30-d exposure of brown trout alevins to aluminum at low pH (4.5, 4.8, 5.4) in low-calcium water (0.4 or 2 mg/L) had no significant effect on mortality or growth but did cause significant reductions in net uptake of calcium, potassium, and sodium and skeletal calcium deposition.

Growth in fish length during early life stages is important to later survival (Braum 1967). Large fish have greater swimming abilities, as reflected by distance covered and velocity attained, than smaller fish of the same age (Webb 1976), allowing them to be more efficient at capturing food and avoiding predators (Bams 1967; Taylor and McPhail 1985). Consequently, Atlantic salmon with reduced growth may have reduced survival potential.

The lack of difference in growth between our two pH control treatments (Table 4, pH 5.5 vs. 6.5) suggests that pH 5.5 in the absence of aluminum is not toxic to young Atlantic salmon. This observation is consistent with the findings of Daye and Garside (1979), Lacroix et al. (1985), and McKee et al. (1989).

Histopathology

Gill pathology is believed to be a very sensitive indicator of sublethal acid stress in fish (McCormick et al. 1989). Our study and those of Leino and McCormick (1993) and Leino et al. (1990) show that this is true for aluminum stress as well. The gross morphology of gills of Atlantic salmon exposed to pH 6.5 or 5.5 in the absence of aluminum resembles that of other salmonids (Kendall and Dale 1979; Jagoe and Haines 1983). Norrgren et al. (1991) similarly found no morphological damage to gills of minnow (*Phoxinus phoxinus*) exposed to pH 5.0 with no aluminum, nor did Karlsson-Norrgren et al. (1986) when studying yearling brown trout exposed to pH 5.5 without aluminum, nor did Lacroix (1989) working with Atlantic salmon parr from rivers with pH of 4.8 to 6.3 and ion exchangeable aluminum concentrations of 1.3 to 43 μg/L. However, even though damage was not induced, histomorphological changes may well have been present, and such changes were found in the gills of fish exposed to low pH (5.5 or lower) in the absence of aluminum or at low aluminum concentrations (Leino and McCormick 1984).

Exposure to 200 μg/L aluminum caused extensive damage to fish gills, especially at pH 5.5. Norrgren et al. (1991) exposed minnows to 150 μg/L aluminum at pH 5 and 6 and found similar morphological damage to the gills, with the damage being more severe at the lower pH. Karlsson-Norrgren et al. (1986) reported similar but qualitatively more severe damage to brown trout exposed to 500 μg/L aluminum at pH 5.5 and minor damage with the same aluminum concentration at pH 7.0. The swelling and fusion of lamellae in the presence of aluminum result from proliferation of respiratory pavement cells and hyperplasia of chloride cells (Karlsson-Norrgren et al. 1986; Jagoe et al. 1987; Evans et al. 1988; Tietge et al. 1988).

Aluminum has been widely reported as a significant toxic factor to fish in acidic waters (see review by Spry and Wiener 1991). The gill is the principal target organ, and death results from a combination of ionoregulatory, osmoregulatory, and respiratory dysfunction (Exley et al. 1991; Wood 1989). The toxicity mechanism is complex and varies with changes in experimental conditions such as pH, hardness, aluminum concentration, and temperature (McCormick and Jensen 1992). For example, Neville and Campbell (1988) exposed juvenile rainbow trout to 76 μg/L aluminum at various pH levels between 4.0 and 6.5 and found a bimodal response in mortality. The peak in mortality at pH 4.5 resulted from electrolyte loss, and the peak at pH 6.1 resulted from asphyxia. The predominant aluminum species present in the test solutions were Al^{3+} and AlF at pH 4.5 and $Al(OH)_2$ and $Al(OH)_4$ at pH 6.1, but the authors concluded that variation in mortality resulted from pH-induced changes at the gill–solution interface rather than changes in aluminum speciation.

We maintained pH constant at two concentrations and aluminum constant but varied aluminum speciation by addition of fluoride (Table 5). The resulting change in aluminum speciation from the ionic aluminum and aluminum hydroxide forms in the absence of fluoride to the fluoride-bound aluminum reduced gill morphological damage at both pH concentrations, especially at the lower fluoride concentration (Figs. 1 and 2). Wilkinson et al. (1990) exposed age-1 Atlantic salmon to 235 μg/L aluminum at pH 4.5 and varied the fluoride concentration from 0 to 228 μg/L. Cumulative mortality over 7 d reached 100% at fluoride concentrations of 124 μg/L and lower, 65% at 171 μg/L fluoride, and 0% at 194 μg/L fluoride. However, in a second experiment when the concentrations of ionic and hydroxide aluminum forms were held constant and the concentration of fluoride aluminum species was increased, mortality also increased, indicating that fluoride-bound aluminum, although less toxic than ionic aluminum, does contribute to toxicity. Parkhurst et al. (1990) exposed brook trout fry to total aluminum concentrations of 4–1087 μg/L and fluoride concentrations of 11–328 μg/L at pH levels ranging from 4.4 to 6.5. In their work, fish survival did not differ among different pH levels. At aluminum concentrations of 112 μg/L, and especially at 325 μg/L, the addition of 111 or 328 μg/L fluoride increased survival, but the increases were relatively minor.

Our results agree with the above findings inasmuch as we found that gill damage was more severe in treatments in which 200 μg/L fluoride was added than in treatments in which 100 μg/L was added (Figs. 1 and 2, Table 5). Increasing the fluoride content of exposure water increased the amount of soluble aluminum so that fish in the higher fluoride treatments were exposed to higher total aluminum concentrations. In the experiments by Parkhurst et al. (1990), addition of fluoride similarly increased the amount of soluble aluminum. Norton et al. (1989) found higher than expected concentrations of soluble aluminum in lakes where fluoride was elevated and that such elevated fluoride concentrations could result from high fluoride content of

bedrock, till, and stream sediments. They concluded that the fluoride contributed to aluminum solubility.

We noted two additional morphological effects in the gills of fish exposed to elevated fluoride. At pH 6.5, but not at pH 5.5, obvious open lesions were present on all gill filaments examined (Figs. 1E–1H). We have not observed such lesions previously, and can find no precedent for them in the literature. The lesions are probably not caused by excess fluoride in the test water because the measured concentrations of free fluoride in the two pH 6.5 treatments were similar to those in the pH 5.5 treatments (114 and 238 μg/L at 100 and 200 μg/L added fluoride at pH 6.5 and 109 and 257 μg/L at pH 5.5). The pores observed in the gills of fish exposed in the high fluoride treatment at pH 5.5 are probably chloride cell apical crypts (Leino et al. 1983). Such crypts are commonly observed in fish subjected to acid and aluminum or beryllium stress (Jagoe et al. 1987, 1993; Leino et al. 1987*a*, 1987*b*).

Low fluoride concentrations may reduce the morphological damage caused by aluminum by reducing the binding of aluminum to gill epithelium. The toxicity of aluminum is postulated to result from binding of aluminum to ligands at the gill surface (Al-L-gill; Neville and Campbell 1988; Wilkinson et al. 1990). The aluminum fluoride species may be less efficient at binding to the gill ligands than ionic or hydroxide species. Indeed, Wilkinson et al. (1990) reported that less aluminum accumulated on gill tissue when fluoride was present. Wilkinson et al. (1993) proposed a fluoride–aluminum–ligand complex that binds to gill tissue. This new ligand complex may be associated with some of the adverse effects observed in our study in the 200 μg/L fluoride treatment at pH 5.5. Wilkinson and Campbell (1993) reported that for given equivalent Al^{3+} concentrations, sorption of aluminum to the gill surface was higher in the presence of fluoride than in its absence, which further indicates the formation of mixed ligand complexes (F-Al-L-gill) at the gill surface. This increased aluminum accumulation at the gill in the presence of fluoride may explain the greater gill damage in our study at 200 μg/L fluoride than at 100 μg/L fluoride (Table 3).

We conclude that small amounts of fluoride (up to 100 μg/L) can reduce gill morphological damage in fish exposed to aluminum in circumneutral acidic waters (Table 2), possibly because fluoride complexes of aluminum bind to gill epithelial surfaces less efficiently than hydroxide complexes. At high fluoride concentrations (>100 μg/L), any reduction in the toxicity of aluminum by binding with fluoride may be more than offset by the increase in soluble aluminum concentration in the water that results from fluoride complexation. Fluoride concentration may, therefore, be an important determinant of aluminum toxicity to fish in acidic waters.

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Spatial implications of a temperature-based growth model for Atlantic cod (*Gadus morhua*) off the eastern coast of Canada

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Abstract: Lengths at age of Atlantic cod (*Gadus morhua*) off the eastern coast of Canada vary up to three-fold among geographic regions, with Georges Bank and the southern Gulf of St. Lawrence being the regions of fastest and slowest growth, respectively. Colour-coded contour maps of length at ages 2–8 (n = 59 518) based on research vessel surveys between 1971 and 1992 clearly showed the spatial variations in size at age. Corresponding maps of July–September bottom water temperature appeared to reflect relative growth rate, whereas those for depth did not. A polynomial expansion of a temperature-based von Bertalanffy growth model appeared to fit the data well, with no residual patterns across most ages, lengths, temperatures, or years. Model predictions were also consistent with reported lengths at age for cod in other regions and countries. Declines in bottom water temperature over the past 5–10 years appear to be at least partly responsible for observed declines in size at age of the cod stock on the eastern Scotian Shelf.

Résumé : La longueur selon l'âge de la morue Atlantique (*Gadus morhua*) sur la côte est du Canada varie par un facteur pouvant aller jusqu'à trois fois entre les différentes régions géographiques. Le Banc Georges constitue la région de croissance la plus rapide alors que le sud du Golfe du St. Laurent est celle de croissance la plus lente. Des cartes couleur des isolignes de la longueur selon l'âge pour les poissons de 2 à 8 ans (n = 59 518) obtenues des relevés scientifiques de 1971 à 1992 montrent clairement la variation spatiale de la taille selon l'âge. Les cartes correspondantes des températures au fond en juillet–septembre semblent correspondre au taux relatif de croissance contrairement à celles de la profondeur. Le développement polynomial d'un modèle de croissance de type von Bertalanffy basé sur la température a donné un bon ajustement des données, sans tendance évidente des résiduels pour la plupart des âges, longueurs, températures ou années. Les prédictions du modèle sont aussi en accord avec les tailles selon l'âge pour d'autres régions et d'autres pays. Selon une autre version du modèle, le déclin de la taille selon l'âge pour la section est du Plateau Néo-Écossais semble être du, au moins en partie, au déclin des températures au fond sur cette zone au cours des 5 à 10 dernières années.

Introduction

Length at age in fish is often highly variable, with the degree of variability increasing with age. For physiological reasons, this observation is expected; fish growth varies with differences in temperature, food consumption, size, and genetic strain, as well as other factors (Brett 1979; Ricker 1979), with the resulting size differences being cumulative with age. As a result, bioenergetics models have had some success in predicting fish length and (or) weight under given environmental conditions (e.g., Madenjian and Carpenter 1991; Arnason et al. 1992). Unfortunately, bioenergetic parameter estimates and measures of food consumption are seldom available in field situations, necessitating an alternative approach. Linear models incorporating environmental terms provide such an alternative (Maceina 1992; Millar 1992; Weisberg 1993) but are more limited in terms of their predictive capabilities. They also fail to take advantage of any spatial patterns in size at age that may exist in the study area (Brandt and Kirsch 1993). Therefore, there appears to be a need for an easily parameterized growth model with predictive capabilities that can be mapped to show large-scale geographic patterns in size at age.

Numerous anecdotal reports have noted large geographic differences in size at age among Atlantic cod (*Gadus morhua*) stocks off the northeastern coast of North America. For example, cod on Georges Bank are widely known for their apparently rapid growth rate, whereas cod from the Gulf of St. Lawrence are equally well recognized for being among the slowest growing of the Atlantic cod stocks. Furthermore, there is considerable spatial heterogeneity in size at age within stocks. For instance, cod caught in research vessel (RV) surveys near the mouth of the Bay of Fundy are consistently larger at age than those on the

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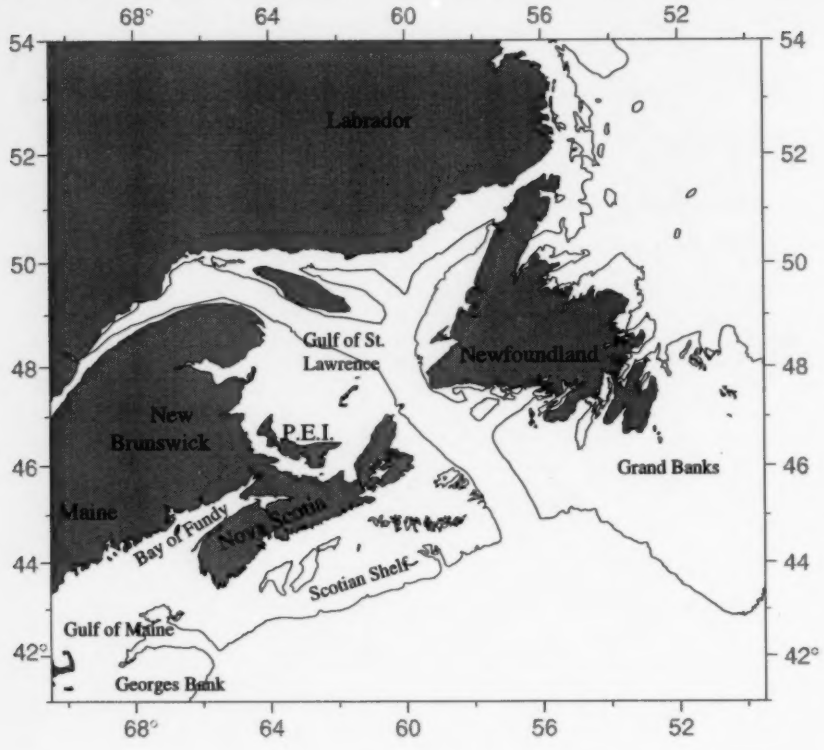
Fig. 1. Map of the study area showing the 200-m bathymetric contour.



offshore banks of Northwest Atlantic Fisheries Organization (NAFO) division 4X, despite the fact that both areas are part of the same stock assessment unit (Campana and Hamel 1992). While regional and temporal variations in cod growth have variously been attributed to the effects of food (Kohler 1964; Braaten 1984), fishing (Hanson and Chouinard 1992), and temperature (Taylor 1958; Jorgensen 1992; Brander 1995), there have been few large-scale examinations of cod growth that incorporate both within-stock and across-stock differences in size at age (but see Brander 1995).

The objectives of this study were to document the large-scale gradients in cod size at age in the southern Gulf of St. Lawrence, the Scotian Shelf, and the Gulf of Maine (Fig. 1) and to develop a simple age–temperature growth model that was consistent with decadal-scale spatial patterns in both size at age and temperature. We conclude by using the model to estimate the impact of long-term cooling on the relative size of cod on the eastern Scotian Shelf.

Materials and methods

Data preparation

All size at age data were derived from RV surveys rather than the commercial fishery to avoid complications resulting from long-term changes in the size selectivity of fishing gear. Length, age, bottom water temperature, and depth data were obtained from July RV surveys conducted by the Department of Fisheries and Oceans for the Scotian Shelf and Georges Bank through the period 1971–1992. The corresponding data from the southern Gulf of St. Lawrence were obtained from September RV surveys. Use of a 19-mm mesh liner in the cod end reduced the probability of any fish extrusion through the net. However, analysis was also restricted to ages 2–8 to minimize the possibility of size-selective availability to the gear throughout the survey region. All cod were measured to the nearest centimetre, and otoliths for age determination subsampled from up to 3 fish per 3-cm length interval in each set (1 fish per 1-cm interval in the Gulf of St. Lawrence since 1985). Estimates of mean length at age for a given set were left unweighted by length frequency because there was little appreciable difference between weighted and unweighted estimates; the slope relating the weighted and unweighted estimates was 0.99, and discrepancies at age were <5%.

The cod length at age data were examined in two ways: using colour-coded contour maps for a visual assessment of spatial pattern and through statistical analysis. The two approaches were intended to complement each other, the first providing an easily grasped overview and the second providing the statistical rigour. However, it is important to note that all hypothesis testing was restricted to the statistical analysis.

Colour-coded contour maps were contoured using Delauney triangles (Watson and Philip 1985) of set-by-set

data averaged over 0.2° squares. Interval scales were extended over the entire range of the data. Because the contour maps were prepared solely for the purpose of assessing spatial pattern, data were aggregated into 11- or 12-year blocks for each map. Visual comparisons between time blocks were then made as assessments of long-term shifts in size at age or temperature.

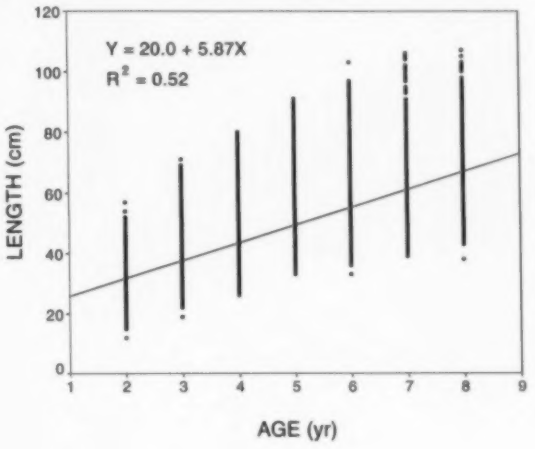
The cod growth model was designed to give equal weight to all geographic regions, irrespective of fish density. Accordingly, a single estimate of mean length at age was calculated for each 0.5° square for each 5- or 6-year time period, on the basis of a mean data density of about 20 observations per cell. Mean temperatures at age were based only on temperatures associated with the capture of cod. While it is unreasonable to assume that temperature at capture represents ambient temperature at earlier ages, this assumption was necessary because of our inability to reconstruct the temperature history of individual fish. Plots of temperature-at-capture versus age and (or) size differed substantially among the five cod stocks (Page et al. 1994; this study) demonstrating that there was no consistent age- or size-related temperature preference. For instance, young cod on the eastern Scotian Shelf and in the Gulf of St. Lawrence tended to be found in warmer water than did older cod, although there was no such tendency off southwestern Nova Scotia or on Georges Bank. While mean annual bottom water temperatures at each site would have been preferred for use as an index of annual temperature exposure by cod, such were not available. Accordingly, we considered it important to use temperature measurements that would not be unduly influenced by short-term seasonal fluctuations. Since bottom water temperatures at shallow stratified sites are more seasonally variable than those at deepwater and well-mixed sites, all collections made at stations of <50 m depth were excluded from subsequent analysis.

Model development

On the basis of known biological principles, there were three features that we thought important to capture in a growth model: inclusion of a temperature term, a gradual reduction in relative growth rate with increasing age, and increasing variance in length at age with age. All of these objectives could conceivably be met through modifications to the von Bertalanffy growth model. As noted by Beverton and Holt (1957), the von Bertalanffy growth model would be expected to reflect the influence of temperature through its effect on the parameter K. Of the two von Bertalanffy model formulations that have been developed to incorporate a seasonal temperature effect, one is based on the definition of a temperature-based growing season (Pitcher and MacDonald 1973), while the other models the parameter K as a smooth and cyclic function of temperature and time (Ursin 1963; Pawlak and Hanumara 1991).

Our interest was in empirically testing for the effect of temperature on the parameters of the von Bertalanffy model. Model building, testing, and diagnosis can be problematic for nonlinear models such as the von Bertalanffy. Therefore, we chose to model growth as a linear function to take full advantage of the diagnostic tools that have been developed for linear models. That is, instead of using

Fig. 2. Linear regression of length on age for all age 2–8 cod collected between 1971 and 1992.



$$[1] \quad L_{age} = L_\infty (1 - \exp(-K(\text{age} - t_0)))$$

where L_{age} is length at age, and L_∞, K, and t_0 are parameters to be estimated, we used a Taylor series expansion of the nonlinear model (see Beverton and Holt 1957, p. 99) to get

$$L_{age} = \beta_0 + \beta_1(\text{age}) + \beta_2(\text{age})^2 + \beta_3(\text{age})^3 + \ldots + \beta_n(\text{age})^n$$

The corresponding terms from the Taylor series expansion are given as

$$\beta_0 = L_\infty(1 - \exp(-Kt_0))$$

$$\beta_1(\text{age}) = L_\infty \exp(-Kt_0)K(\text{age})$$

$$\beta_2(\text{age})^2 = \frac{-L_\infty \exp(-Kt_0)K^2(\text{age})^2}{2}$$

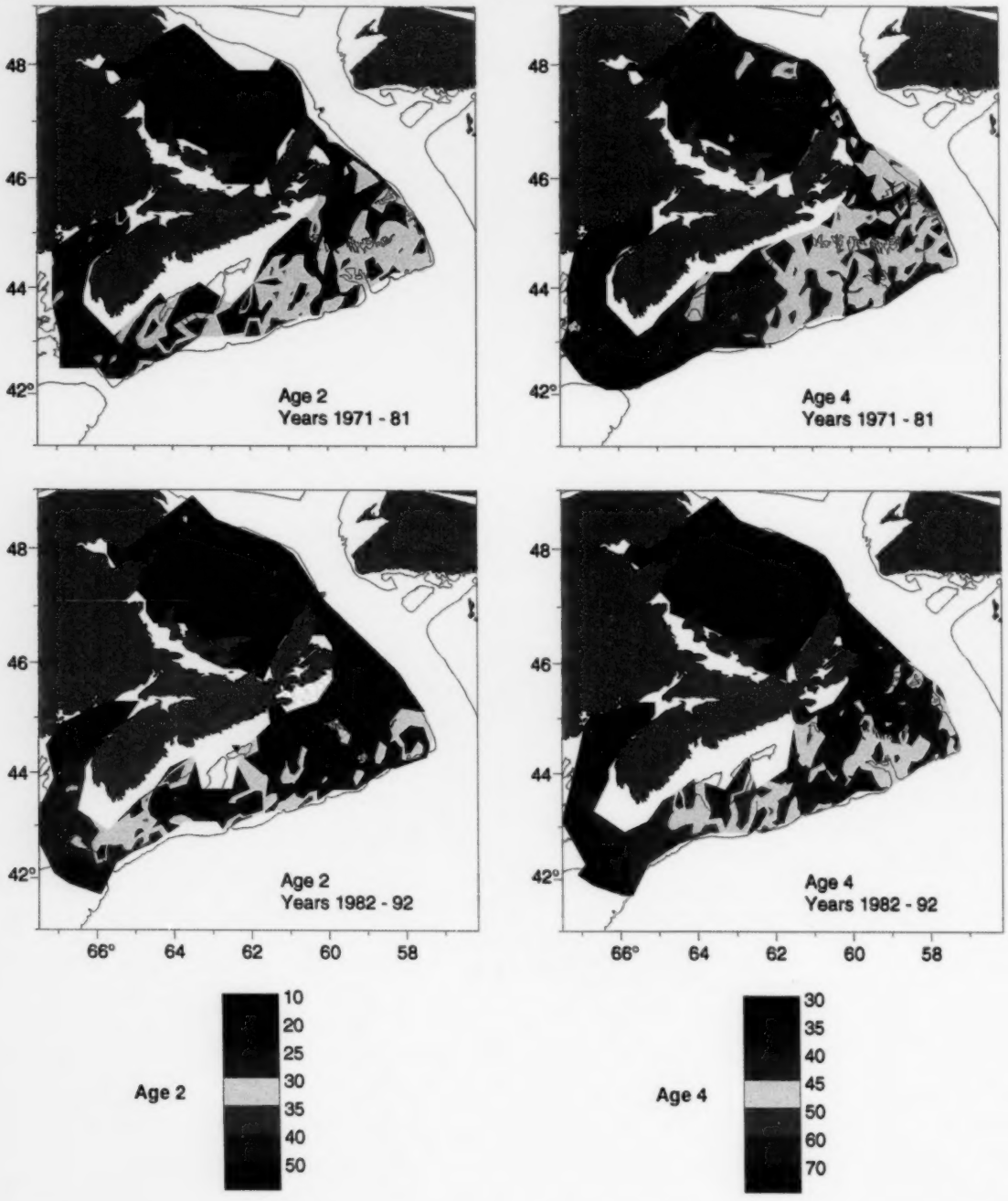
$$\beta_3(\text{age})^3 = \frac{L_\infty \exp(-Kt_0)K^3(\text{age})^3}{6}$$

etc.

Initial plots of the mean length at age against the associated standard deviation suggested that the standard error increased with the mean, but that the coefficient of variation remained constant. Models with Gaussian errors require constant standard error (variance). A constant coefficient of variation is more characteristic of a gamma distribution. Therefore, we used a generalized linear model (McCullagh and Nelder 1989) with a gamma error and identity link. The gamma model is more efficient than simply assuming a lognormal distribution and analyzing the log-transformed data (Firth 1988).

All parameter estimates were estimated using the iteratively reweighted least-squares algorithms in S-PLUS (STATSCI 1993). Temperature was included in the model as a simple additive term and as an interaction term with each of the age polynomial terms. The significance of all terms in the model was tested using the analysis of deviance approach and χ^2 test discussed in McCullagh and Nelder (1989).

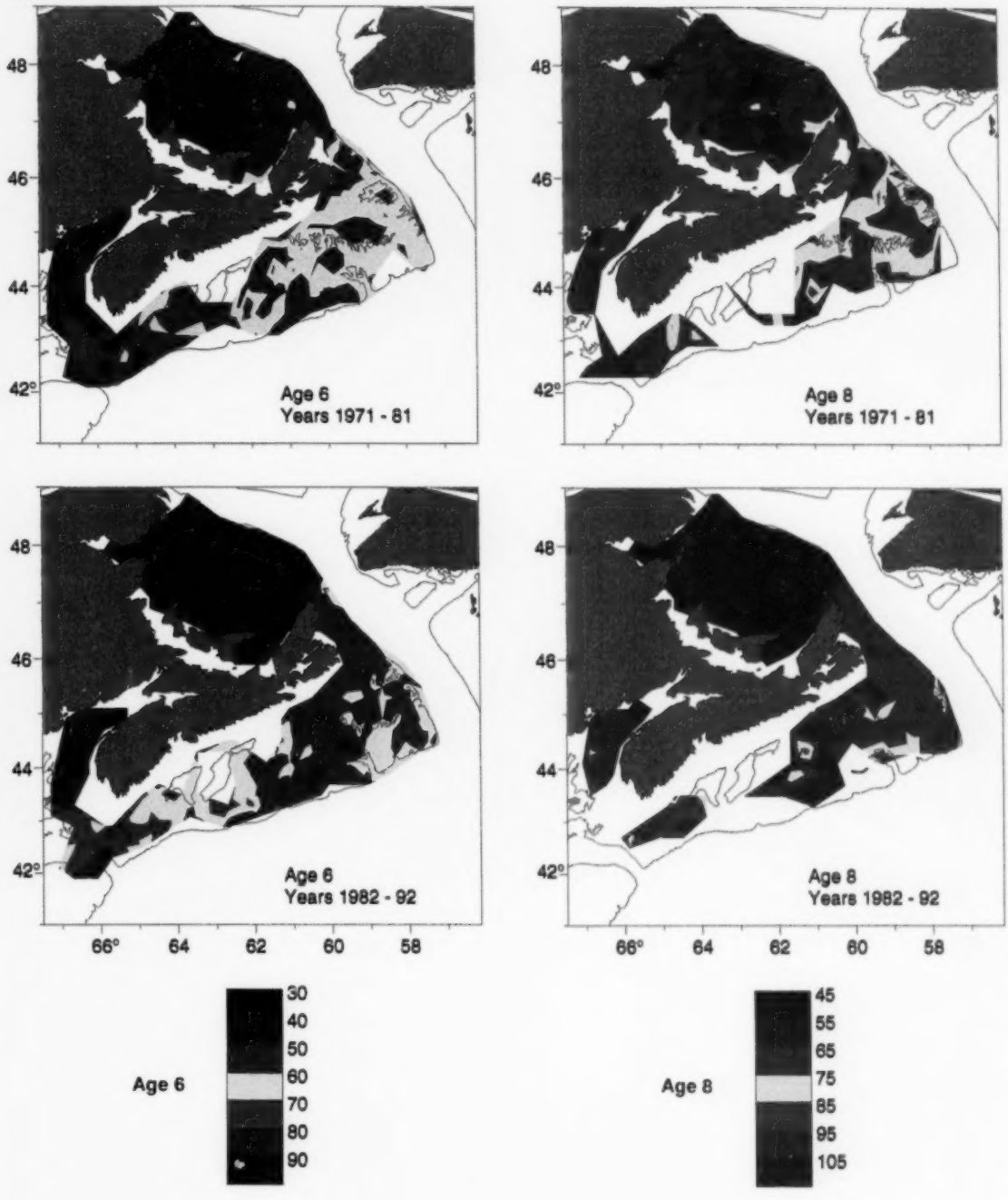
Fig. 3. Colour-coded contour maps of cod showing spatial patterns in relative size (cm) at each of four ages (ages 2, 4, 6, and 8) and two periods (1971–1981 and 1982–1992). The 200-m bathymetric contour is indicated.



Results

A total of 59 518 cod length at age observations were collected between 1971 and 1992. A simple regression of length on age highlighted the expected increase in variance with age and relatively low explained variance (52%) (Fig. 2). However, the distribution of the regression residuals was clearly nonrandom across ages, predicted values, and stocks, indicating that a simple regression was unsuitable for describing these data. When viewed as colour-coded contour maps

Fig. 3 (*concluded*).



(Fig. 3), the relative size of cod at each age varied substantially and coherently among regions, thus explaining at least part of the variability in the length– age regression. Indeed there was at least a three-fold difference in size at age between cod from the southern Gulf of St. Lawrence and Georges Bank at all ages. In general, size at age decreased from north to south along the Scotian Shelf, although the decline was not clearly linked to latitude: cod in the Bay of Fundy were up to 50% larger than their counterparts at the same latitude on the Scotian Shelf.

Fig. 4. Colour-coded contour maps of July bottom water temperatures (September in the Gulf of St. Lawrence) as recorded in research vessel surveys, along with the 200-m bathymetric contour. Temperatures (°C) represent all those recorded, not just those associated with cod catches.

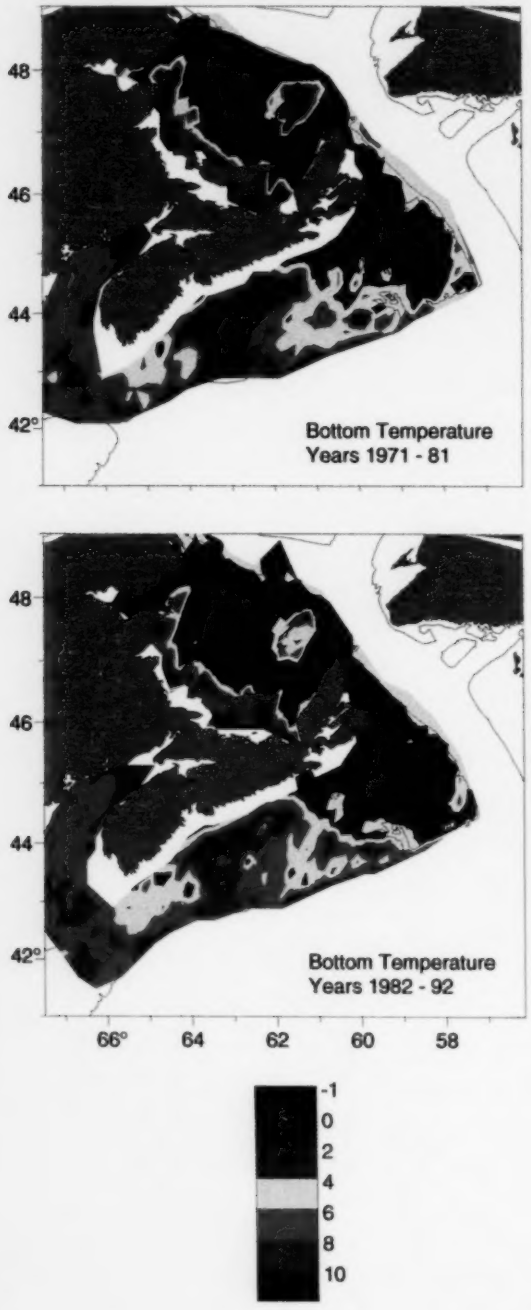
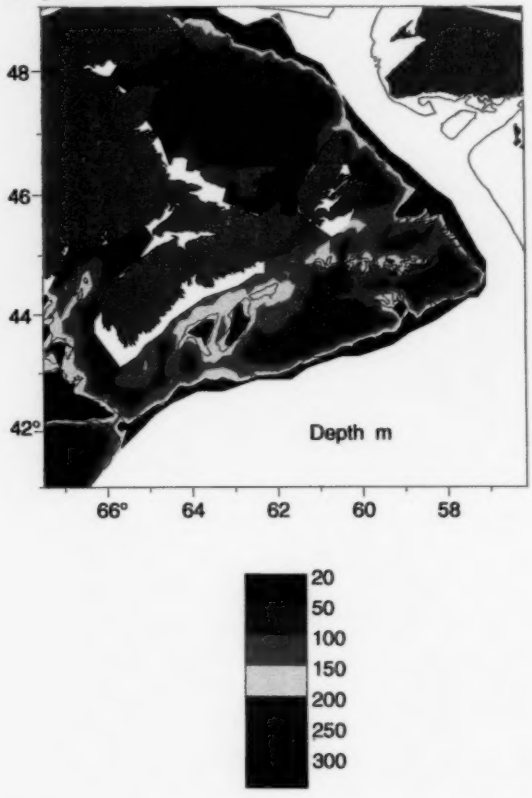


Fig. 5 Colour-coded contour map of depth (m) in the study area.



Comparison of the 1971–1981 period with that of 1982–1992 indicates that there has been a long-term shift in size at age and that the direction of the shift has varied with the area. The most recent decade has seen a shift towards smaller size at age of cod in both the southern Gulf of St. Lawrence and the eastern Scotian Shelf. There was little apparent change in size for the regions further to the south.

The spatial patterns of bottom water temperature at the time of the survey (Fig. 4) were similar to those of cod size at age. In general, the southern Gulf of St. Lawrence was relatively cold, while the waters of the Gulf of Maine and the deepwater basins of the Scotian Shelf were relatively warm. Anomalously warm areas in the southern Gulf of St. Lawrence were associated with shallow (<50 m) stratified sites (Fig. 5), thus justifying their removal from the growth modelling section of the study. Long-term shifts in bottom water temperature were not clearly evident at the decadal level of comparison (Fig. 4), although comparisons of 5-year periods showed that the Gulf of St. Lawrence and eastern Scotian Shelf cooled during the mid to late 1980s.

Temperature was a significant covariate of length at any given age (ANCOVA, $p < 0.001$). When aggregated by 2° intervals, temperature was associated with an apparent

Fig. 6. Relationship between length and age of cod when weighted uniformly by geographic area (see Methods). Solid lines represent regression lines fitted to 2° temperature categories, indicating an apparent increase in growth rate associated with an increase in temperature.

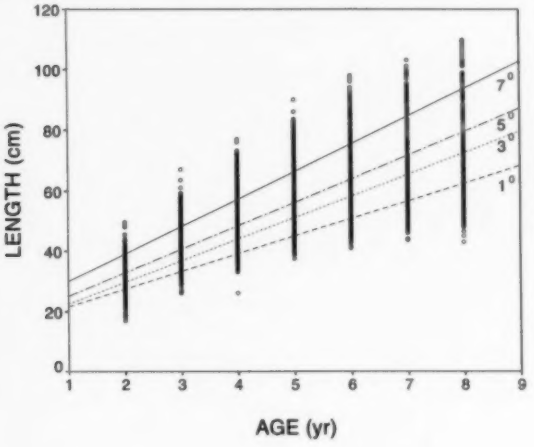
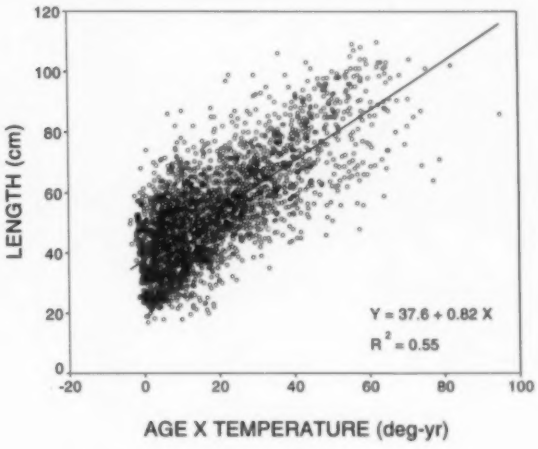


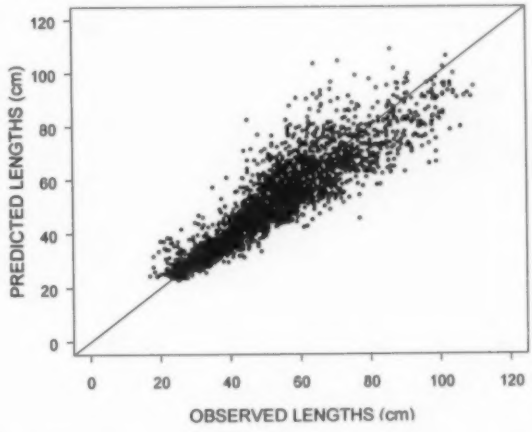
Fig. 7. Linear regression of length on the product of age and temperature (degree-years). While statistically significant, this regression did not explain appreciably more of the variance than did the regression of length on age.



increase in growth rate of cod (Fig. 6). To determine if the relationship could be explained as simply as through the use of cumulative growing years, degree-years (based on a point estimate of temperature for each year) were regressed on length (Fig. 7). The relationship was highly significant but explained no more of the variance than did the simple regression of age on length. In addition, the residuals were not distributed randomly across ages, years, temperatures, or stocks. Thus, such a simple model was not sufficient to explain much of the observed variability in cod size at age.

A Taylor expansion of the modified von Bertalanffy equation using a gamma error provided a much better fit to the length at age data than did the simpler models considered previously. Only the lower order terms of the Taylor expansion proved to be statistically significant. All possible submodels were tested against the full model defined as having a second-order polynomial in age, with temperature as a main effect and included in interaction with all of the age terms. The best submodel was a simple model of $L = 1 + \text{age} + \text{temperature} + \text{age} \times \text{temperature}$ (Table 1). However, when tested against a third-order version of the same full model, the best submodel lacked a significant age × temperature interaction term (Table 1). There was no objective means for selecting between these two submodels. Therefore, we initiated an empirical forward-stepping model fitting procedure, in which the same set of age and temperature terms described above were added hierarchially to a simple mean model, along with a stock term. The resulting set of significant terms included all of those derived from the Taylor expansion quadratic submodel described above but with the addition of a significant stock term (Table 2). Therefore, the submodel resulting from the quadratic model was accepted as best, while the stock effect in Table 2 was interpreted as a reflection of geographic differences in temperature seasonality. Parameter

Fig. 8. Observed versus predicted values of cod length at age based on a Taylor expansion of a temperature-based von Bertalanffy growth model. The deviance residuals of the model were randomly distributed across all predicted values, time periods, temperatures, and ages, with the exception of the very smallest and largest fish. The 1:1 line is indicated.



estimates for the age, temperature, and age × temperature terms in the Taylor expansion and forward-stepping models were similar, despite the additional presence of the stock term in the latter model. Temperature × stock interactions were also significant, reflecting the different patterns of temperature seasonality in the different stocks. No obvious patterns were evident in the residual plots from the Taylor expansion submodel, with the exception of size underestimation of the largest fish (Fig. 8).

Geographic variations in the degree of model fit were viewed by mapping the deviance residuals at age (Fig. 9).

Table 1. Hierarchial screening of terms for the Taylor expansion of the temperature-based von Bertalanffy growth model and parameter estimates resulting from the accepted quadratic submodel.

Hierarchial screening for Taylor expansion of temperature-based von Bertalanffy growth model

Terms	df	Deviance	*P* value	
			Cubic	Quadratic
Full model (cubic)	2923	47.681	—	—
Full model (quadratic)	2925	47.796	—	—
Reduced models				
Age^3 × temperature	2924	47.775	0.759	—
Age^3	2925	47.796	0.943	—
Age^2 × temperature	2926	47.808	0.988	0.916
Age^2	2927	50.669	0.559	0.238
Age × temperature	2928	57.765	0.073	0.007
Temperature	2929	124.122	<0.001	<0.001
Age	2930	335.683	<0.001	<0.001

Parameter estimates resulting from the accepted quadratic submodel

Parameter	Coefficient	Standard error
Intercept	13.08	0.393
Age	5.84	0.092
Temperature	0.56	0.098
Age × temperature	0.51	0.024

Note: The full cubic model is length = 1 + age + temperature + age × temperature + age^2 + age^2 × temperature + age^3 + age^3 × temperature, with gamma distributed errors. The full quadratic model is similar, but lacks the cubic terms. *P* value refers to a χ^2 statistic for difference between the deviances of the full and reduced models. The terms listed under reduced models refer to terms sequentially removed from the full model.

Table 2. Hierarchial screening of terms for a forward-stepping empirical model of cod growth.

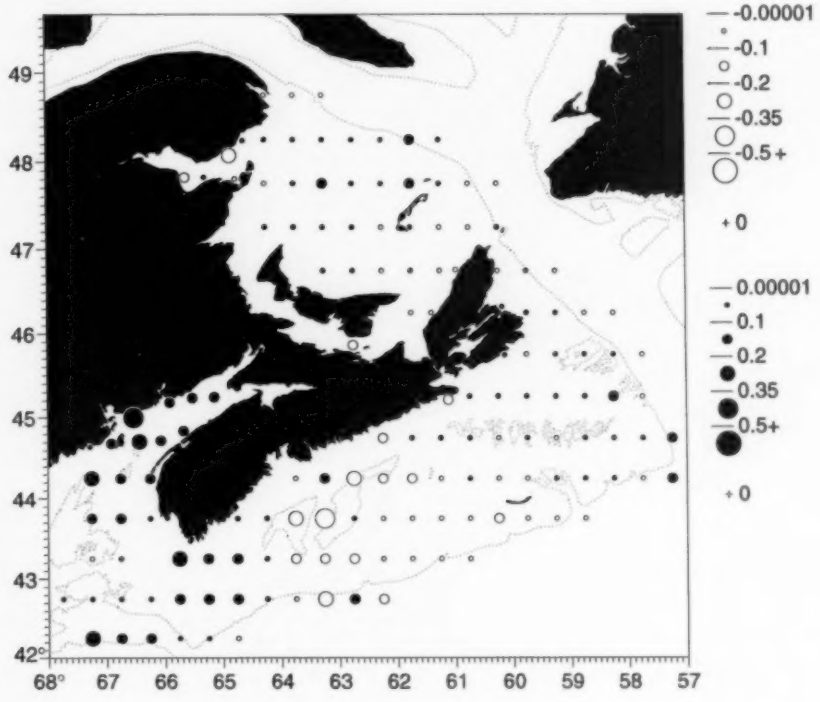
Terms	df	Change in deviance	*P* value
Null			
+ age	1	211.5	<0.001
+ temperature	1	66.4	<0.001
+ stock	6	82.0	<0.001
+ age + stock			
+ temperature	1	2.5	0.110
+ age × temperature	1	8.1	0.004
+ age + temperature			
+ stock	6	18.2	0.006
+ age × temperature	1	7.1	0.008
+ age + temperature + stock + age × temperature	1	6.9	0.009

Note: The NAFO divisions corresponding to the stock term are as follows: 4T, 4Vn, 4Vs, 4W, 4X offshore banks, 4X Bay of Fundy, and 5Z.

All of the age-related residuals showed a similar geographic pattern, with relatively small residuals in the Gulf of St. Lawrence and Scotian Shelf, positive residuals in the southern portion of the range, and negative residuals in the warm, deep basins of the Scotian Shelf. In other words, predicted lengths were smaller than observed in the south and larger than observed in the deep basins. Model fit elsewhere was relatively good. Spatial patterns in deviations from predicted values appeared to cross stock boundaries and thus were associated with regions rather than populations.

Using the temperature-parameterized growth model, we wished to answer the following question: could declining temperatures on the eastern Scotian Shelf have been responsible for the observed decline in length at age of cod in the region (Mohn and MacEachern 1994)? Bottom water temperatures (Fig. 10) and relative growth rates on the eastern Scotian Shelf have declined since the 1980s, whereas temperatures and relative growth rates further south have not. Using the observed lengths of age 2 cod as a starting point (smoothed with a 3-year running mean), we calculated annual growth increments for cohorts of cod from NAFO division 4Vs on the basis of observed 4Vs bottom water temperatures and the parameterized growth model. Note that these data were not identical to those used in fitting the model, since the latter used 5-year time blocks across the entire survey region, while the model predictions were based on annual temperatures in only 15% of the region. The predicted lengths of age 8 cod tended to reflect the

Fig. 9. Spatial distribution of the deviance residuals from the age- and temperature-based growth model for age 4 cod across all year blocks. In all age categories, fish length tended to be underestimated in the warm southern portion of the study area, and overestimated in the warm deep basins.



lengths that were actually observed, at least qualitatively (Fig. 11). In particular, the timing of the pre-1980 increase and the post-1980 decrease in length at age was well predicted by the model, although the rate of decline was underestimated. The fit of the model was not as good when fixed or predicted lengths at age 2 were used as starting points, suggesting that variations in growth during the first 2 years of life carried through to the adult stage. Temperature may also have played a role in the growth of cod less than 2 years old, since temperature and observed length at age 2 were significantly correlated ($p < 0.05$).

Discussion

The large-scale spatial gradient in cod size at age along the eastern coast of Canada appears to be largely explicable on the basis of temperature alone. These results are consistent with previous growth studies on cod, in which a significant effect of temperature has been noted (Taylor 1958; Nakken and Raknes 1987; Loeng 1990; Jorgensen 1992; Brander 1995). This is not to say that temperature effects on fish growth are necessarily direct; various workers have reported that growth rate increased with temperature only in the presence of sufficient rations, presumably through the intermediary of increased consumption (Kohler 1964; Cui and Wootton 1988; Brown et al. 1989; Waiwood et al. 1991). While our analysis would not have been able to differentiate between temperature and food effects if they were highly correlated in space, the documented importance of temperature on fish growth (Brett 1979; Ricker 1979; Houde 1989), in conjunction with the observed spatial gradient in temperature, suggests that food limitation alone was not responsible for the large-scale patterns in growth rate in the study area. Griffiths et al. (1992) noted that the growth in length of Arctic ciscoes (*Coregonus autumnalis*) was considerably more sensitive to temperature than was growth in weight; the latter was more strongly influenced by food availability and would probably be more apparent at smaller spatial and temporal scales.

The advantages of mapping patterns in fish growth are not restricted to ease of visualization (Brandt and Kirsch 1993). While the colour-coded contour maps certainly made it easier to see spatial patterns in relative size, it was the maps of the model residuals that highlighted the inadequacies of the growth model. Some discrepancy is unavoidable, because cod are not fixed particles in space. Indeed, cod from the Gulf of St. Lawrence migrate well outside the Gulf each winter, although growth during this period is relatively minor (Anonymous 1994). Seasonal temperature cycles also differ substantially among regions, suggesting that the length of the growing season is almost certainly dependent upon region. For example, bottom water temperatures in southwest Nova Scotia often stay

Fig. 10. Bottom water temperatures (°C) on the eastern Scotian Shelf and in the Bay of Fundy as recorded on RV surveys between 1971 and 1992. There have been no long-term shifts in temperature in the Bay of Fundy, but temperature has declined on the eastern Scotian Shelf since the mid-1980s.

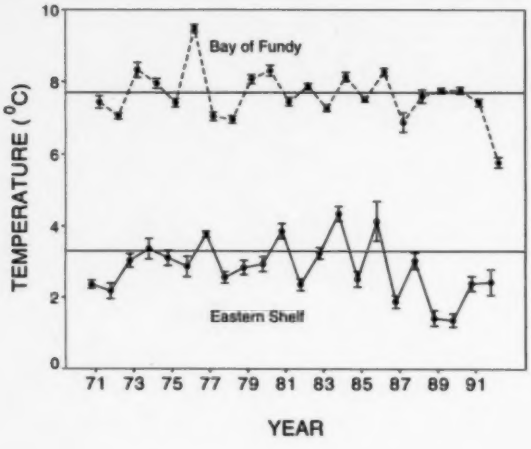
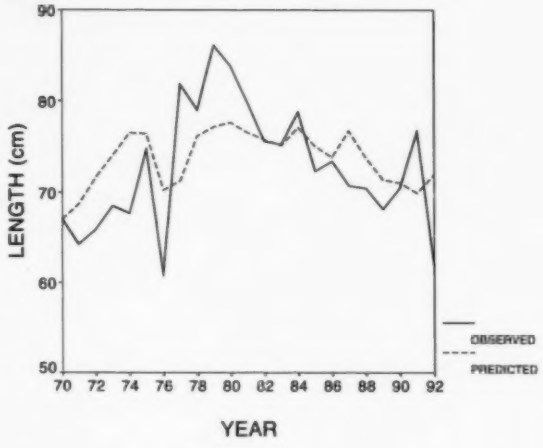


Fig. 11. Observed and predicted lengths of age 8 cod (cm) on the eastern Scotian Shelf between 1971 and 1992. Model predictions were based on the parameters of Table 1 and observed age-specific bottom water temperatures in NAFO division 4Vs, using smoothed observed lengths at age 2 as starting points for the growth trajectories of each cohort.



above 2°C during the winter (Smith 1989), while much of the shallow water of the southern Gulf of St. Lawrence is below 0°C (Strain 1988). Thus, it is reasonable to expect a longer growing season in the southern portion of the range than in the northern portion, and hence growth underestimation (positive residuals) in the former. This effect was also apparent as a statistically significant stock effect in the growth model. Cumulative temperature within a year (akin to growing days) might be a more useful measure of growth temperature than is a point estimate of temperature during the summer. Presumably, there are regional variations in food availability that also affect the relative growth rate of cod. The negative residuals associated with the warm deep basins of the Scotian Shelf indicated the presence of undersized cod, pointing to the possibility of food limitation in those areas. Indeed, this model may be well suited to identifying regional variations in scope for growth (sensu Brandt and Kirsch 1993).

The age–temperature growth model presented here not only accounted for much of the observed variation in size at age in the waters off of eastern Canada, it provided reasonably accurate predictions of cod size at age in other regions. Jonsson (1965) reported mean August bottom water temperatures of 8.5, 6.7, 4.4, and 3.6°C in four hydrographic provinces off of Iceland, associated with age 6 cod of 81.2, 77.8, 68.7, and 65.0 cm, respectively. The predictions of the growth model lay within 7% of each of these values. Predictions of the length of age 4–6 cod in the Barents Sea were accurate to within 5% of observed values (Nakken and Raknes 1987), on the basis of the August bottom water temperatures of Loeng (1990). Similarly, the length of age 5 cod off the coast of Labrador (NAFO division 3K) was estimated to be 48.4 cm, versus an observed length of 50 cm (May et al. 1965). Finally, the length of age 5 West Greenland cod was estimated to within 3% of observed values (Hermann and Hansen 1965), on the basis of July bottom water temperatures (Elizarov 1965). But despite the relative accuracy of these and other length at age predictions, the growth model is conceptually flawed. It is well known that growth rate is a function of not only environmental variables but fish size as well (Braaten 1984). In its current form, however, the model would predict that a shift in temperature would result in an annual increment that is a function of an age–temperature interaction rather than a size–temperature interaction. In other words, should a 4-year-old cod that had been reared at 2°C increase in length the same amount as a much larger 4-year-old cod that had been reared at 8°C, if the two were then placed in the same environment? On the basis of observations of growth trajectories in other species (Makinen and Ruohonen 1992), the answer would appear to be no, yet to our knowledge, all published linear growth models fail to make that prediction. Bioenergetic growth models should be able to avoid this problem, but face the additional constraints of obtaining measures of food availability and parameter estimates in wild fish.

Long-term shifts in water temperature appear almost certain to produce concomitant shifts in the growth rate of cod. More specifically, the decline in water temperature appears to explain at least some of the decline in size at age of eastern Scotian Shelf cod since the mid-1980s, with heavy exploitation of the faster growing fish possibly accounting for some of the remainder. Reduced water temperatures may also account for declines in the growth rate of cod in the Gulf of St. Lawrence and off Newfoundland. Similar reasoning has been invoked to explain long-term shifts in the relative size of cod elsewhere in the world (Nakken and Raknes 1987; Loeng 1990; Jorgensen 1992; Brander 1995). Thus, it seems odd that Frank et al. (1990)

referred only to growth effects owing to increased winter feeding as being among the global warming effects predicted to affect cod stocks. However, where stocks are near the limit of their range, it may be that temperature effects on growth will prove to be secondary to other factors, as has been reported for fish stocks on Georges Bank (Ross and Nelson 1992).

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Examination of assumptions critical to body component indices: application to the giant scallop *Placopecten magellanicus*

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Abstract: Allometric relations of gonadal mass to shell height for the scallop *Placopecten magellanicus* from Baie des Chaleurs, Gulf of St. Lawrence, varied between maturing and fully mature individuals. We describe a technique for separating the two groups. We calculated gonosomatic indices for fully mature scallops and found that the three assumptions critical to body component indices, that (*i*) allometric relationships for the body component being studied and the reference component do not change over the size range of animals studied, (*ii*) slopes in the allometric growth equation for the studied and reference components are similar, and (*iii*) mass of the reference component does not change over time, were violated. We examined two alternative methods for calculating body component indices. The first applied a correction factor to the gonosomatic index and the second directly scaled the component studied to maximum shell height. We recommend the latter. We identified spawning (drops in gonadal mass) between successive dates using a modified Jonckheere rank test, which is appropriate for time-series data.

Résumé : Les relations allométriques entre le poids de la gonade et la hauteur de la coquille pour le pétoncle géant *Placopecten magellanicus* de la Baie des Chaleurs, golfe du St. Laurent, variaient entre les individus en maturation et ceux pleinement matures. Nous décrivons une technique permettant de différencier les deux groupes. Nous avons calculé les indices gonosomatiques pour des pétoncles pleinement matures et montré que les prémices critiques à l'utilisation des indices corporels, soit (*i*) les relations allométriques pour la composante corporelle étudiée et la composante de référence ne changent pas sur la gamme des tailles étudiées, (*ii*) les pentes de la relation de la croissance allométrique pour la composante étudiée et celle de référence sont similaires, et (*iii*) la masse de la composante de référence ne change pas dans le temps, n'ont pas été respectées. Nous avons examiné deux méthodes alternatives à l'utilisation des indices corporels. La première consistait à appliquer un facteur de correction à l'index gonosomatique et la deuxième à standardiser directement la composante étudiée par la hauteur maximale de la coquille. Nous recommandons cette dernière. Nous avons identifié les périodes de pontes (baisse dans la masse gonadique) entre dates successives en appliquant un test de rang de Jonckheere modifié, plus approprié à des données de séries temporelles.

Introduction

Indices are used to study changes in body components over time, or between populations, and are often applied to invertebrates (Giese and Pearse 1974) and fish (Ricker 1975). They involve scaling the studied component to a reference component to eliminate variations owing to size. For example, when reproductive cycles are being followed, gonadal indices are often obtained by dividing gonadal mass (or volume) by animal mass (or volume).

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When there are variable rates of increase in the mass for different body components with increasing body size, growth is allometric (Huxley 1924; Teissier 1948). If the slope (*b*) describing the allometric relationship of body component mass to size equals 3, volume or mass increase in proportion to size and growth is isometric; however, the slope is usually greater or less than 3 (Reiss 1989; Safran 1992). Body component indices assume that (*i*) allometric relationships for the body component being studied and the reference component do not change over the size range of animals studied, (*ii*) the slopes in the allometric growth equation for the studied and reference components are similar, and (*iii*) the mass of the reference component does not change over time. Although these assumptions should be tested prior to using body component indices (Gonor 1972; deVlaming et al. 1982; Cayré and Laloë 1986; Packard and Boardman 1988; West 1990), this is seldom done.

Violation of assumptions can lead to increased variation in indices, thereby reducing the probability of detecting significant changes in gonadal or somatic body components, or can cause erroneous conclusions that changes have occurred when they have not.

The aim of the present study was to establish an appropriate method to follow changes in the mass of body components. We explored this subject because we were studying spawning in the giant scallop *Placopecten magellanicus*, and found that previously employed gonosomatic indices do not respect the three underlying assumptions about allometric relationships. Using data for giant scallops, we demonstrate problems inherent in the use of conventional indices and then describe alternative methods for scaling the mass of body components to body size. Further, we present a statistical technique to test for decreases in gonadal mass, as would occur during spawning, and discuss how to test for changes in other components over time.

Differences between juveniles and adults in the relation of body component mass to body size are known for many taxa, for example sea urchins (Gonor 1972), crabs (Somerton 1980), whelks (Martel et al. 1986), and fish (deVlaming et al. 1982). Thus, the two groups are usually treated separately. When studying changes in gonadal indices over time to determine when spawning occurs, it is preferable to sample only fully mature animals for which gonadal mass is maximal. We describe an objective method for determining the size at full maturity.

Materials and methods

Sampling of scallops

The scallop populations studied were at Carleton (13 m in depth, 48°04.8′N, 66°06.6′W) and Gascons (26 m in depth, 48°09.86′N, 64°55.58′W) on the north shore of the Baie des Chaleurs, southwestern Gulf of St. Lawrence, Canada. To compare how the relationships among body components varied with size and between sites, large samples of scallops were collected by divers using self-contained underwater breathing apparatus (SCUBA) shortly before spawning (when gonadal size was maximal). At Carleton, we obtained 249 scallops, ranging from 51 to 153 mm in shell height (dorsal to ventral axis) on 9 and 24 June and 6 and 31 July 1984, and at Gascons, 234 scallops, from 29 to 142 mm in height, were collected on 27 July and 2 and 5 August 1988. To compare how relationships among body components were affected by spawning, an additional 176 scallops, measuring 65–148 mm in height, were collected after spawning at Carleton in September and October 1984. Finally, to evaluate whether the masses of potential reference body components were stable over the reproductive period, approximately 30 mature scallops (about 15 males and 15 females measuring 100–152 mm in shell height) were collected at Carleton at frequent intervals from 1 July to 3 October 1986. This included a period of growth prior to spawning, spawning, and the beginning of gonadal recovery after spawning.

All scallops collected were covered with seaweed and kept in an insulated box until they were dissected (within 3 h of collection). For each scallop we measured shell height (to the nearest 0.1 mm) and total mass (soft tissue and shell) after draining excess water from the mantle cavity. The scallop was then dissected to determine the sex and the wet mass of the shell, gonad, muscle (quick and catch adductor muscles), and remaining viscera (digestive gland, gills, mantle, and foot). The latter were weighed to the nearest 0.01 g, after allowing each component to drain on paper towelling for about 5 min. We used wet mass in this study because it was readily determined. Robinson et al. (1981) showed that for giant scallops the drop in gonadal mass during spawning is indicated equally well by wet and dry measures of gonadal mass.

Theoretical analysis

In the first part of the study, we compared how allometric relationships changed among body components, with size, between sites, and before and after spawning. Shell height was used as the reference measure for scallop size, because unlike the soft tissues, it does not shrink under adverse environmental conditions (MacDonald and Thompson 1985*a*). Further, the maximal growth rate reported for 90-mm scallops is about 1 mm/month (MacDonald 1986), so that little growth occurred during the sampling period of this study.

The relation between the mass of a particular body component and overall size is typically allometric and may be expressed as

$$[1] \quad Y = a \cdot H^b$$

where Y is the body component mass, H is the shell height, a is the intercept, and b is the slope. Both mass and shell height were log transformed (base 10) to achieve linearity ($\log Y = \log a + b \cdot \log H$).

The sex was readily distinguished for nearly all of the scallops in the size range sampled. Allometric relationships change with size. When studying changes in gonadal indices, it is preferable to use fully mature animals. Thus, we separated scallops into two groups, maturing individuals for which the gonad rapidly increases in mass with increasing shell height, and fully mature individuals for which the slope of gonadal mass to shell height is less. To determine the line of best fit for each group, we applied a method that minimizes the residual sum of squares between maturing and fully mature scallops. The size at full maturity was determined as follows. Using the log-linear relation of gonadal mass to shell height, we calculated the residual sum of squares starting with the smallest two scallops (45–50 mm in height) and then recalculated it repeatedly, each time increasing the size of the group by adding the next larger scallop. At each iteration, we also calculated the residual sum of squares for the remaining larger scallops. When the sum of the residual sum of squares for small and large scallops at each iteration was plotted against shell height, the size at full maturity was defined by the minimal value in the U-shaped curve. This was determined from a fitted second-degree polynomial.

We also examined if sexes could be combined in body component indices when studying fully mature scallops. The body components examined were gonadal mass and the potential reference components, total mass and muscle mass. The log-transformed masses were compared by analysis of covariance (ANCOVA), using shell height as the covariate.

In the second part of the study, we examined the assumption that body component indices were independent of size. Such indices usually compare the mass of one body component (Y_1) with the mass of a reference component (Y_2). On the basis of the allometric relation (eq. 1) of mass to body size (H), the ratio of the mass of the two components in the index is defined as

$$[2] \quad \frac{Y_1}{Y_2} = \frac{a_1 \cdot H^{b_1}}{a_2 \cdot H^{b_2}}$$

where the subscript 1 refers to the body component of interest and the subscript 2 to the reference component. When the slopes in the relationships between the two body components are equal ($b_1 = b_2$), the index is independent of size and the ratio in eq. 2 is reduced to

$$\frac{Y_1}{Y_2} = \frac{a_1}{a_2} = a_3$$

where a_3 is the mass of the studied component relative to that of the reference component. It is assumed that the slopes are similar and the reference mass is stable over time. However, if the reference mass is not stable over time, then the calculated index value (a_3) may not be representative of the relative changes in the studied body component, even if the slopes are equal ($b_1 = b_2$). Thus, the accuracy of the index as an indicator of changes in the studied component is reduced.

If the slopes of the two body components are not equal ($b_1 \neq b_2$), then the ratio of the two components (eq. 2) becomes

$$[3] \quad \frac{Y_1}{Y_2} = \left(\frac{a_1}{a_2}\right) \cdot H^{b_1 - b_2}$$

This index is dependent on size because of the effect of the difference in slopes. We tested the assumption that body component indices are independent of size for fully mature scallops by plotting the gonosomatic index (drained gonadal mass to total mass) against shell height for the size range of scallops collected prior to spawning.

We examined two techniques to eliminate the effect of size on the index. The first, the corrected gonosomatic index, was obtained using eq. 3, by dividing the index by shell height raised to $b_1 - b_2$, the difference between the slopes of the studied and the reference components, i.e.:

$$\text{corrected gonosomatic index} = \frac{\text{gonadal mass}}{\text{total mass}} \cdot \text{shell height}^{b_1 - b_2}$$

The second method, the gonadal mass index, consisted of directly scaling gonadal mass to shell height, i.e.:

$$[4] \quad \text{gonadal mass index} = a = \frac{Y}{H^b} \cdot k = \frac{\text{gonadal mass}}{\text{shell height}^b} \cdot k$$

where b is the slope used in the regression of gonadal mass to shell height for fully mature animals and k is a constant determined by the units adopted for Y and H, to obtain a value greater than zero. The gonadal mass index for an individual is equivalent to the intercept a. The same technique can also be applied in studying changes in mass of other body components.

Finally, we examined the assumption that the reference mass is stable over time by analyzing variations in allometric relationships for various body components for fully mature scallops collected between July and October 1986 at Carleton. We applied ANCOVAs to the log-transformed data and verified that distributions of scaled body component mass conformed to assumptions of normality and homogeneity of variance. The covariate was shell height. ANCOVA was used solely to determine whether the slopes were equal or not, because this test cannot be used to interpret the differences in adjusted means when the slopes are heterogeneous (Huitema 1980). The slope b was obtained from the log-linear relation of body component mass (Y) to shell height (H) for each of the 15 dates. The slopes were pooled if they were homogeneous during the different dates ($p > 0.05$). For each body component, the common slope, or that obtained at each date when the slopes were different, was then used to calculate the body component mass index for each scallop (eq. 4). This mass was then converted to a mass for a standard scallop measuring 125 mm in shell height ($Y' = a \cdot 125^b$). This size of scallop represented the mean size collected in 1986 (122.2 ± 10.3 mm (mean ± SD), range 100–152 mm, $n = 483$) and thus minimized possible error associated with the application of a common slope. To graphically illustrate changes in mean body component mass over time, the mean and 95% confidence intervals were calculated from the scaled mass (Y') of the scallops collected on each sampling date.

Statistical considerations

Changes in body component mass over time were compared using Kruskal–Wallis tests applied to scaled values for 125-mm scallop. The null hypothesis H_0 ($\mu_1 = \mu_2 = \ldots = \mu_k$) is that mean ranks of k independent samples are from the same population, and the global hypothesis H_1 is that at least one μ_i is different from the others (Siegel 1956). When the analysis indicated differences between dates (k samples), Dunn's post-hoc multiple comparison test, which is appropriate for unequal sample size and variance, was applied to the mean ranks. Gonadal mass values on different dates were compared using the nonparametric Jonckheere test for k (>2) independent samples with an ordered alternative (H_1) hypothesis (Jonckheere 1954; Siegel 1956; Capéraà and Van Cutsen 1988). The test statistic (T) is generally used in time-series studies and is calculated as follows:

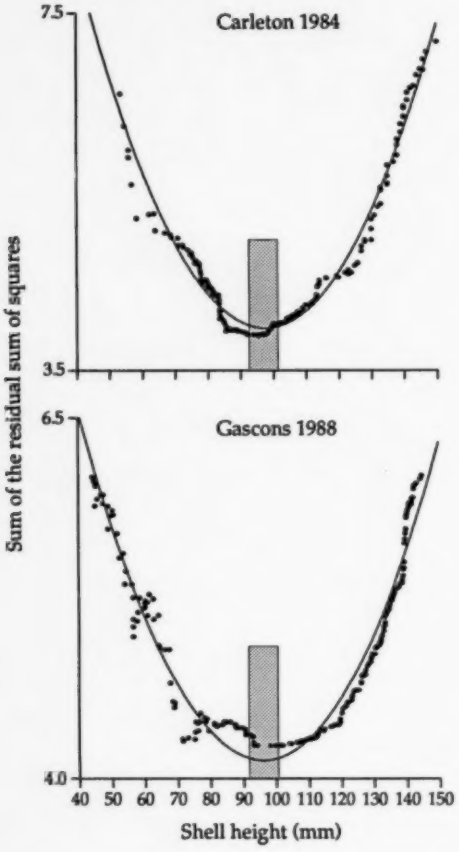
$$T = \sum_{i<i'} U_{ii'}$$

where $U_{i\,i'}$ is the Mann–Whitney statistic calculated for the ranks between the two samples i and i'. We modified the statistic to specifically compare observations on successive dates (date$_i$ with date$_{i+1}$) to identify downward changes (Appendix), as would occur with spawning. A Kruskal–Wallis test (rank sums) would be inappropriate for testing an ordered alternative hypothesis (H_1) of the following form:

$$H_1: \mu_1 \geq \mu_2 \geq \ldots \geq \mu_k$$

because H_0 would be rejected whenever there was sufficient difference between the mean ranks, irrespective of

Fig. 1. Size at maturity. The residual sum of squares was calculated at each increment in size using the log-linear relation of gonadal mass to shell height, starting with the two smallest scallops and then recalculating it repeatedly, each time adding the next larger scallop. At each iteration, we also calculated the residual sum of squares for the remaining larger individuals. The minimum value of the sum of the two residual sums of squares against shell height, as indicated by a fitted second degree polynomial applied to the data, indicates the size at full maturity (shaded bar).



their order (Capéraà and Van Cutsen 1988). For example, when studying reproductive events, samples taken prior to and after a major spawning would always cause differences in ranks. For all tests a significance level of α = 0.05 was used. The modification of the Jonckheere test results in the α value being divided by $k - 1$ samples, rather than $k(k - 1)/2$ samples, as in post-hoc multiple comparisons.

Results

Size at full maturity

At both Carleton and Gascons, the plot of the sum of the residual sum of squares against shell height for scallops collected just prior to spawning indicated that maturing and fully mature scallops should be separated at about 95 mm in shell height (Fig. 1). When the sexes were analyzed separately, the curves were less regular because of the reduced number of points; nevertheless, a similar separation between maturing and fully mature scallops was indicated. This method of determining the size by minimizing the residual sum of squares was more objective than visually selecting inflection points from curves of gonadal mass or gonosomatic index to shell height (Figs. 2C and 3A).

Differences between sexes

Allometric relationships of total, muscle, and gonadal mass to shell height for maturing scallops (<95 mm) differed between sexes at Carleton but not at Gascons (Table 1). At both Carleton and Gascons, no differences between the sexes were detected in regression coefficients for the various body components for fully mature scallops collected during the period of maximal gonadal size (late June and July at Carleton and late July and August at Gascons). When the analysis at Carleton was repeated including the 9 June sample, the slopes of the gonadal mass to shell height were the same but the gonadal mass (adjusted mean) of females was lower ($p < 0.05$). This indicated a delay in attaining the gonadal maximum for females at Carleton. In subsequent analyses of fully mature scallops, the sexes were combined.

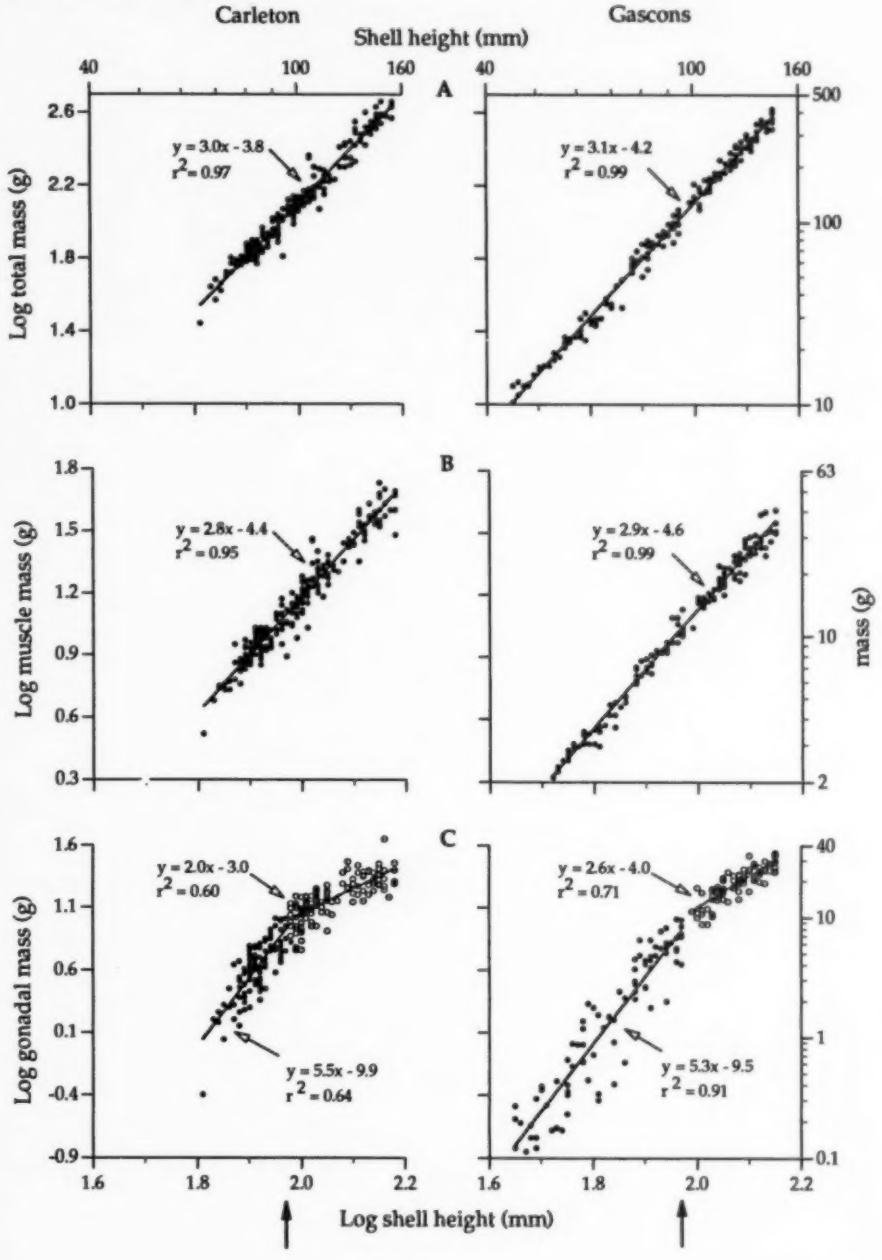
Changes in the relative mass of body components with size

At both Carleton and Gascons one regression line (r^2 = 0.95–0.99) can be used to describe the relationship between both total and muscle mass to shell height for maturing and fully mature animals (Figs. 2A and 2B, Table 2). As the previous analysis of size at maturity indicated, the relation of gonadal mass to shell height varied between maturing and fully mature scallops. Slopes of 5.5 and 2.0, respectively, were obtained at Carleton, and 5.3 and 2.6, respectively, at Gascons (Fig. 2C, Table 2). To examine if allometric relationships vary between locations for fully mature scallops (>95 mm), we compared the components for Carleton and Gascons. The slopes of regressions of total and muscle mass to shell height were similar, but the adjusted means of these body components were larger at Carleton (Table 3).

We next considered the assumption implicit to employing body component indices, that the slopes of studied and reference components are similar (homogeneous). In half the comparisons between various pairs of body components for fully mature scallops, this assumption was not valid (gonadal mass compared with total and muscle mass at Carleton, and muscle mass compared with total mass at Gascons; Table 4).

The slopes for the mass of the muscle, gonad, viscera, and total mass to shell height were not significantly affected by spawning (Table 5). However, the probability of homogeneous slopes was close to the critical level for the gonad (p = 0.06). This indicates that changes in slope could affect the gonadal mass index. Nevertheless, it is advantageous to establish a common slope because this permits us to use the index to monitor the relative drop in gonadal

Fig. 2. Relationship to shell height of (A) total mass, (B) muscle mass, and (C) gonadal mass for scallops from Carleton and Gascons. Size at full maturity (>95 mm) is indicated by the arrow below the abscissa.



mass during spawning. (During spawning, regression analyses cannot be applied because of the simultaneous occurrence of ripe and spent individuals.) Selection of a standard-sized scallop from near the mean size of individuals sampled would reduce possible effects of changes in slope on the adjusted gonadal mass. Adjusted means of reference components were not stable during the reproductive period, there being a decrease in muscle and total mass, and an increase in viscera mass shortly after spawning (Table 5).

Fig. 3. Relationship to shell height of (A) gonadal mass/total mass (gonosomatic index), (B) corrected gonosomatic index ((gonad/total)/$H^{b_1-b_2}$), and (C) gonadal mass index, (gonad mass/H^b) for scallops from Carleton and Gascons. The corrected slope, $b_1 - b_2$, the difference between the slopes of gonadal mass and total mass to shell height (from Figs. 2C and 2A, respectively), is indicated in B and the slope for the relationship of gonadal mass to shell height (from Fig. 2C) is indicated in C.

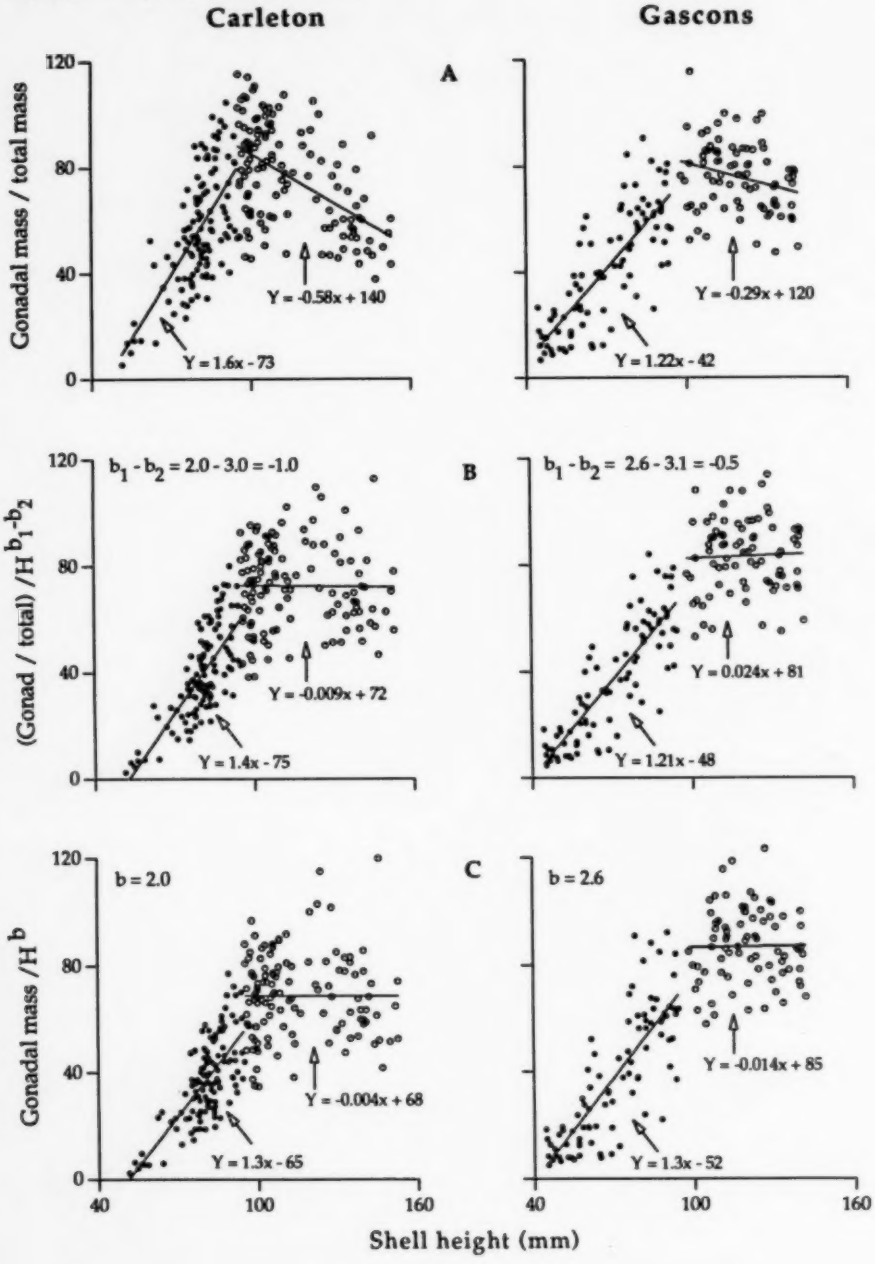


Table 1. Variance ratios (F) and their associated probabilities obtained from separate ANCOVAs comparing males and females for total, muscle, and gonadal mass (shell height as covariate; $p < 0.0001$) for scallops collected prior to spawning at Carleton (1984) and Gascons (1988) (see Fig. 2).

Body component	Regression coefficient	Carleton		Gascons	
		F	p	F	p
Maturing scallops (<95 mm)					
Total	Slope	16.28	0.0001	0.61	0.44
	Adjusted mean			0.009	0.94
Muscle	Slope	7.94	0.006	0.25	0.62
	Adjusted mean			3.27	0.08
Gonad	Slope	37.09	0.0001	0.24	0.89
	Adjusted mean			0.29	0.59
Fully mature scallops (>95 mm)					
Total	Slope	0.05	0.82	<0.0001	0.99
	Adjusted mean	3.33	0.07	0.023	0.88
Muscle	Slope	2.33	0.13	1.32	0.25
	Adjusted mean	0.03	0.86	5.30	0.023
Gonad	Slope	0.88	0.35	2.61	0.11
	Adjusted mean	3.76	0.06	1.49	0.22
Fully mature scallops at Carleton (including the sample from 9 June)					
Gonad	Slope	0.89	0.35		
	Adjusted mean	9.85	0.002		

Note: All values were log transformed.

Table 2. Variance ratios (F) and their associated probabilities obtained from separate ANCOVAs comparing maturing and fully mature (>95 mm) scallops for total, muscle, and gonadal mass (shell height as covariate; $p < 0.0001$).

Body component	Regression coefficient	Carleton		Gascons	
		F	p	F	p
Total	Slope	0.05	0.82	3.19	0.08
	Adjusted mean	0.10	0.75	1.09	0.29
Muscle	Slope	2.17	0.14	3.55	0.06
	Adjusted mean	0.44	0.51	0.56	0.45
Gonad	Slope	215.97	0.0001	20.63	0.0001
	Adjusted mean				

Note: All values were log transformed (data presented in Fig. 2).

Table 3. Variance ratios (F) and their associated probabilities obtained from separate ANCOVAs comparing muscle, gonad, and total mass for fully mature (>95 mm) scallops between Carleton and Gascons (shell height as covariate; $p < 0.0001$).

Body component	Regression coefficient	F	p
Muscle	Slope	1.20	0.27
	Adjusted mean	32.87	0.0001
Gonad	Slope	6.78	0.009
	Adjusted mean		
Total	Slope	0.02	0.89
	Adjusted mean	11.22	0.0001

Note: All values were log transformed (data presented in Fig. 2).

Independence of size of gonosomatic indices

Our data showed that gonosomatic indices only partly removed the effect of size. At both Carleton and Gascons, the gonosomatic index increased with shell height in maturing scallops (Fig. 3A). The relationship changed markedly in fully mature scallops; the gonosomatic index decreased with increasing size at both sites (Fig. 3A). This was because for fully mature scallops, the slope of gonadal mass to shell height was less than that for total mass to height (Figs. 2A and 2C). The effect of size accounted for 19.7% of the variation (r^2) in the gonosomatic index of mature scallops at Carleton and 6.7% at Gascons (Table 6).

We then used eq. 3, dividing the gonosomatic index by shell height raised to $b_1 - b_2$, to correct for the differences in the slopes between gonadal mass and total mass ($2.0 - 3.0 = -1.0$ for Carleton and $2.6 - 3.1 = -0.5$ for Gascons). Then, at both sites the slope of the relation between the corrected gonosomatic index and size was not different from 0 ($p > 0.05$; Fig. 3B, Table 6). Finally, we also obtained a gonadal index that was independent of size by dividing gonadal mass by shell height raised to the power b (eq. 4), the slope in the relation of gonadal mass to shell height (Fig. 3C; $b = 2.0$ for Carleton and $b = 2.6$

Table 4. Variance ratios (F) and their associated probabilities obtained from separate ANCOVAs comparing body components (shell height as covariate; $p < 0.0001$) for fully mature (>95 mm) scallops collected at Carleton and Gascons.

Body components compared	Regression coefficient	Carleton		Gascons	
		F	p	F	p
Muscle and total	Slope	2.52	0.11	6.13	0.014
	Adjusted mean	2396.00	0.0001		
Gonad and muscle	Slope	17.45	0.0001	0.02	0.88
	Adjusted mean			896.00	0.0001
Gonad and total	Slope	30.92	0.0001	3.03	0.08
	Adjusted mean			694.00	0.0001

Note: All values were log transformed (data presented in Fig. 2).

Table 5. Variance ratios (F) and their associated probabilities obtained from separate ANCOVAs comparing muscle, gonad, viscera, and total mass for the period before and after spawning for fully mature (>95 mm) scallops collected at Carleton in 1984 (shell height as covariate; $p < 0.0001$).

Body component	Regression coefficient	F	p	Adjusted mean (g)	
				Before	After
Muscle	Slope	1.01	0.32		
	Adjusted mean	39.43	0.0001	21.09	18.66
Gonad	Slope	3.47	0.06		
	Adjusted mean	738.33	0.0001	13.65	4.02
Viscera	Slope	0.05	0.82		
	Adjusted mean	686.06	0.0001	21.75	46.45
Total	Slope	2.12	0.15		
	Adjusted mean	50.55	0.0001	179.47	170.22

Note: The adjusted mean masses were obtained from ANCOVAs (standard error of the means varied from 1.01 to 1.04). All values were log transformed.

for Gascons). At both sites, the slope of this gonadal mass index to size was not different from 0 ($p > 0.05$; Table 6).

Temporal changes in body components

Slopes of regressions of muscle mass, muscle and viscera mass combined, and shell mass to shell height for the 15 collection dates were homogeneous (Table 7). We thus used the pooled slope for muscle mass ($b = 2.4$), muscle and viscera mass combined ($b = 2.7$), and shell mass in converting values for individual scallops to that of an individual measuring 125 mm ($Y' = a \cdot 125^b$). In contrast, because the slopes for viscera and total mass varied over time (Table 7), slopes for each sampling date were used to convert values for each scallop to that of a standard scallop. Slopes for gonadal mass were homogeneous before and after spawning, but not during spawning. As would be expected because of gamete loss, the adjusted mean gonadal mass was smaller after spawning. Because of the variation in gonadal size during spawning (scallops ranging from ripe to spent), a regression line could not be fitted to the data. We imposed the pooled slope ($b = 2.0$) for samples taken before and after spawning in converting gonadal mass values for individual scallops to that of a standard scallop. The mean of the standardized values thus illustrated the transition from prespawning to postspawning condition. Allometric relationships of gonadal mass to all other body components (muscle, viscera, muscle plus viscera, shell, and total mass) were compared for the dates before spawning when the distributions for gonadal mass values respected the assumptions of normality. All slopes were different from that for the relation of gonadal mass to shell height (ANCOVA, $p < 0.05$).

For most body components, scaled mass for a 125-mm scallop changed markedly over time (Fig. 4). As expected, the greatest change was for gonadal mass. The mean was relatively stable during July and then dropped by 33% between 5 and 8 August (equivalent to 9.6 g). A 22% increase (4 g) occurred in the following week and then additional spawning activity occurred between 19 and 22 August and between 2 and 9 September. No differences were detected over time for shell mass, such that it might be considered an appropriate reference component in examining changes in other components. All other components (muscle, viscera, muscle plus viscera, and total mass) varied even during the 3-month sampling period and thus would cause distortions if used as reference

Table 6. Coefficients of determination (r^2), variance ratios (F), and their associated probabilities obtained from separate ANOVAs testing the relation of the gonosomatic index (GSI), corrected gonosomatic index (GSI/$H^{b_1-b_2}$), and gonadal mass index (gonadal mass/H^b) to shell height for significant departures from zero, for fully mature (>95 mm) scallops collected at Carleton and Gascons (data presented in Fig. 3).

	Carleton			Gascons		
Index	r^2	F	p	r^2	F	p
GSI	0.197	29.35	<0.0001	0.067	5.71	0.02
GSI/$H^{b_1-b_2}$	0.005	0.55	0.46	0.0004	0.03	0.85
Gonad/H^b	0.006	0.69	0.41	0.0002	0.01	0.91

components in examining changes in gonadal mass during the reproductive period.

To examine how a reference mass increases the variation in gonosomatic indices, thus potentially reducing the power of a statistical test to detect changes over time, we compared plots of the coefficients of variation of the gonosomatic index (using muscle mass as a reference component) with that of the gonadal mass index (Fig. 5). For most of the samples, more variation was observed when using the gonosomatic index. This can be attributed to allometric differences between the muscle (b = 2.4) and gonad (b = 2.0), and to the fact that the adjusted muscle mass varied over time (Fig. 4B, Table 7). Both indices increased during spawning events because of the presence of scallops in varying stages from ripe to spent.

Table 7. Variance ratios (F) and their associated probabilities obtained from separate ANCOVAs comparing masses of various body components among k dates at Carleton in 1986 (shell height as covariate; $p < 0.0001$; data presented in Fig. 4).

Body component	Regression coefficient	F	p
Gonad	Slope	1.43	0.18
	Adjusted mean	168.00	0.0001
Muscle	Slope	0.49	0.95
	Adjusted mean	14.81	0.0001
Viscera	Slope	2.52	0.001
	Adjusted mean		
Muscle + viscera	Slope	1.55	0.08
	Adjusted mean	18.96	0.0001
Shell	Slope	1.47	0.11
	Adjusted mean	0.94	0.52
Total	Slope	1.86	0.025
	Adjusted mean		

Note: Pre- and post-spawning dates (k = 9) were analyzed for gonadal mass, whereas all dates (k = 15) were analyzed for other components. All values were log transformed.

Discussion

Size at full maturity

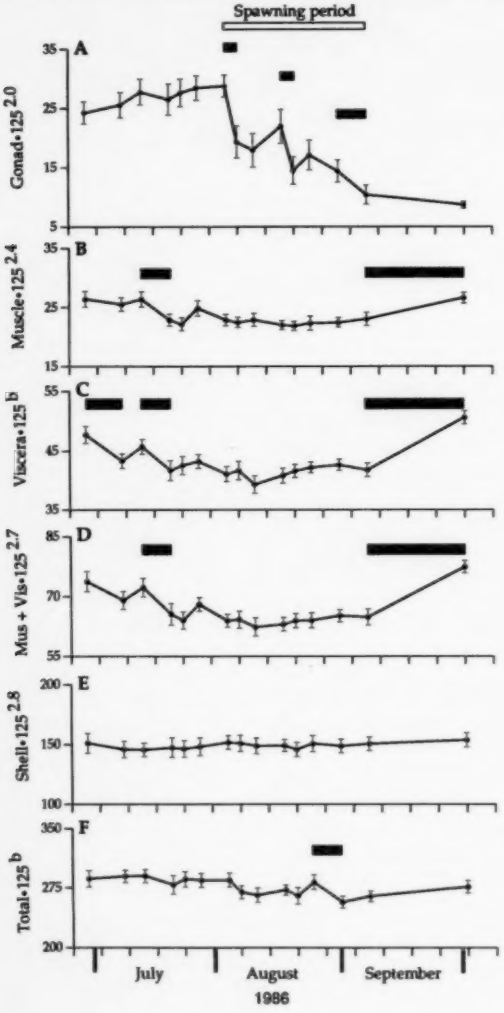
Our examination of relations amongst body components for giant scallops in Baie des Chaleurs showed that assumptions implicit in the use of body component index ratios were not respected. The relation of gonadal mass to size varied between maturing and fully mature scallops so that imposing a single regression line would markedly increase the variance explained by the model. Most studies of scallops and clams recognize the change between maturing and fully mature individuals and treat them separately; however, in studies where the size range examined may include both groups (Peterson 1983; Paulet et al. 1988), gonadal mass could be overestimated for small and large animals and underestimated for sizes near the inflection point. Whereas Peterson (1983) suggests that gonadal production in clams may be reduced in very large animals because of senescence, we found no evidence of this for giant scallops, once the index was corrected (Figs. 3B and 3C).

The size at full maturity was about 95 mm in Baie des Chaleurs. Many studies indicate the size range used to calculate gonosomatic indices (Robinson et al. 1981, range 79.8–163.0 mm; Beninger 1987, >60 mm; Barber et al. 1988, range 85–137 mm), but have not quantitatively evaluated the size at maturity. When assessing seasonal changes in reproductive condition, it is desirable to exclude individuals that have not attained full maturity. Parsons et al. (1992) indicate that giant scallops in Passamaquoddy Bay, New Brunswick, reach full maturity at about 80 mm, which is smaller than for scallops in Baie des Chaleurs. We suggest that size at maturity be determined from analysis of a large sample size (>150 individuals) before studies on reproduction are initiated. A relatively even sampling of different sizes is needed; otherwise, the minimum of the sum of residual sum of squares tends to be skewed toward the sizes that are under sampled. Although variations in body component mass may be further reduced by restricting the size range of fully mature individuals sampled, this is often not feasible because of the number of animals available. Size at maturity does not appear to vary with time, since samples collected at Gascons and Carleton in 1992 (M. Claereboudt, Laval University, Québec, Que., personal communication) indicated the same size at maturity as observed in this study.

Differences between sexes

Most workers combine the sexes to calculate body component indices (Robinson et al. 1981; MacDonald and Thompson 1985*b*; Barber et al. 1988) or estimate production (MacDonald 1986), because differences between gonadal index means or allometric relations were not observed. Our study and previous data (collected at four sites over 8 years, Bonardelli 1994) show that although gonadal mass is similar for males and females during the gonadal maximum in Baie des Chaleurs, it is less for females before the gonadal maximum (9 June in this study). Differences between the sexes prior to the gonadal peak are also reported by Beninger (1987) and Parsons et al. (1992). The reason for the difference between the sexes is probably that oogenesis is delayed compared with spermatogenesis

Fig. 4. Changes in mean mass of various body components for a standard scallop at Carleton measuring 125 mm in shell height (1 July to 3 October 1986). Vertical bars represent 95% confidence limits. For the relationships of viscera and total mass to shell height, the slopes (b) were not homogeneous over time ($p < 0.05$, Table 7) and thus were calculated for each date. Decreases in gonadal mass (Jonckheere test, $p < 0.05$) and changes in the mass of other body components (Dunn's test, $p < 0.05$) are indicated by horizontal bars.



(Naidu 1970; Beninger 1987; Parsons et al. 1992). Ovarian and testicular size vary markedly in many species (Martel et al. 1986; Boivin et al. 1986; Hamel et al. 1993), so that differences between sexes must be examined prior to pooling them. If sexual differences in development do exist at certain periods, changes in gonadal mass should be examined separately.

Selection of a reference component in body component indices

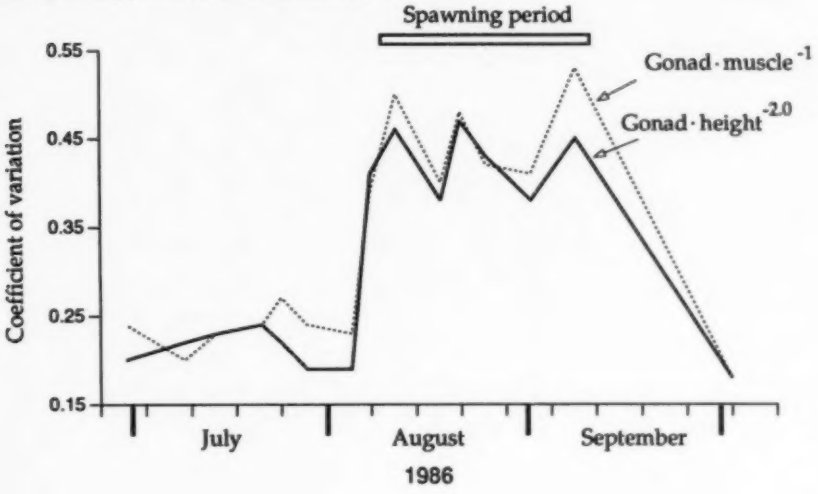
A frequent problem in using body component indices is that the assumption of equal slopes (rate of increase in mass with size) for the studied and reference components, is invalid. This condition was not met for the scallop. We further showed that most standard reference components were not appropriate as denominators for body component indices because they were not stable over time. Shell mass might be a suitable reference component for studying spawning of giant scallops in that neither its slope nor its mass vary over the reproductive period. However, its slope (b = ~2.8) differs from that of gonadal mass (b = 2.0). Thus, if shell mass is employed as a reference component, the gonosomatic index should be adjusted for the difference in slopes (eq. 3). A disadvantage to using shell mass as a reference component is its great mass compared with other components. Even slight variations in this reference component could provoke false changes in the index.

We suggest that a better method is to directly scale the body component being studied to size, as indicated by shell dimension. This method is applicable when temporal variations in shell height are negligible. The rate of bivalve shell growth generally changes with age (Seed 1976) and, further, may be uncoupled with the growth of soft body components (Hilbish 1986; Harvey and Vincent 1990). Thus, when shell height is used as the covariate, scaled mass can decrease because of increases in shell length rather than actual changes in mass. One method to eliminate the effect of shell growth when calculating body component mass is to measure shell height (as covariate) at the point where the annual growth mark is formed (Hilbish 1986; Borrero and Hilbish 1988). Whereas this method may be useful when studying seasonal changes in growth, it is probably unnecessary when studying large animals where shell growth is negligible. Direct scaling of mass to size has been successfully applied in a number of studies of reproductive output (Baxter 1983; Robinson et al. 1981; MacDonald and Thompson 1985*a*, 1985*b*; Harvey and Vincent 1991). For organisms where body dimensions are readily measured, the use of body component indices is not optimal. Greater precision is obtained by measuring only the mass of body components of interest and a body dimension.

Statistical examination of temporal changes in body components

Huitema (1980) recommends pairwise comparisons of adjusted means using the Dunn–Bonferroni or the Bryant–Paulson generalization of Tukey's honestly significantly different test for multiple comparisons of regression equations. We did not use these procedures for comparing temporal changes in the relation of body component mass to size because of the variation in slopes. Rather, we converted to scaled values for a standard scallop and then applied a Kruskal–Wallis test followed by Dunn's procedure. This was appropriate because it does not require homoscedasticity. We did not apply either the post-hoc Games–Howell (Kirk 1982) or Tukey–Kramer's procedures because neither parametric procedure would have corrected for the loss in degrees of freedom caused by the transformation

Fig. 5. Changes in the coefficient of variation for the gonosomatic index (gonadal mass/muscle mass) and for the gonadal mass index (gonadal mass·height$^{-2.0}$) for the period from 1 July to 3 October 1986 at Carleton. Most of the variation occurred during the spawning period (horizontal bar; Fig. 4).



to scaled values. The Tukey–Kramer's procedure was also not appropriate because it requires equal sample size and variance.

Because we were interested in gonadal decreases, as would occur during spawning, we applied a unidirectional Jonckheere test to examine changes in gonadal mass. In our sampling at Carleton, only the first two spawnings would have been detected had the bilateral post-hoc Dunn's test been applied following a global Kruskal–Wallis test. We suspected there may have been spawning activity between 2 and 9 September because we observed a marked gonadal drop between 5 and 8 September in a nearby site (Bonaventure) (Bonardelli 1994). Our application of the Jonckheere test is the first use of a unidirectional statistical test to document decreases in gonadal mass during spawning. This test is not appropriate in examining changes in other body components where increases or decreases are of interest.

We developed the gonadal mass index because we were interested in determining spawning times in giant scallops on the basis of samples periodically collected over the summer from several locations (Bonardelli et al. 1996). The scaled mass method is also appropriate as a step in quantifying changes in space and time in (*i*) reproductive output, (*ii*) energy allocation, and (*iii*) concentrations of biochemical components. Consideration of allometric changes in mass of the gonad is particularly critical when estimating reproductive output by dominant cohorts over months or years.

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Appendix

Modification of Jonckheere's test

To test H_0: $\mu_1 = \mu_2 = \ldots = \mu_k$ against H_1: $\mu_1 \geq \mu_2 \geq \ldots \geq \mu_k$, Jonckheere (1954) proposed the test statistic (T) that applies to the study of time series, and is calculated as follows:

$$T = \sum_{i<i'} U_{ii'}$$

where $U_{ii'}$, is the Mann–Whitney statistic calculated for the ranks (R) between the i and i' samples, where

$$U_{ii'} = \sum_{j=1}^{n_i} R_{ij}^{i'} - \frac{n_i(n_{i+1})}{2}$$

The critical region of the test is of the form ($T \geq c$), where c depends on α.

The modified statistic

The statistic was modified to compare observations at successive time points where evidence was sought of a downward change, as would occur when gametes are released during spawning. H_0: $\mu_1 = \mu_2 = \ldots = \mu_k$ is tested against the alternative ordered hypothesis of the form H_1: $\mu_i \geq$

μ_{i+1}, $\forall\, i \in \{1, 2, \ldots, k-1\}$, which takes account of the ranks between two successive periods (date_i with date_{i+1}) for $k-1$ periods to be compared. Thus, the Mann–Whitney statistic is calculated for the ranks between the i and $i+1$ samples. The modified test statistic is defined as

$$T = \sum_{i=1}^{k-1} U_{i,i+1} = \sum_{i=1}^{k-1}\left(\sum_{j=1}^{n_i} R_{ij}^{(i+1)} - \frac{n_i(n_{i+1})}{2}\right)$$

Calculation of the mean (T) for H_0:

$$E_0(T) = \sum_{i=1}^{k-1} E(U_{i,i+1}) = \sum_{i=1}^{k-1} \frac{n_i(n_{i+1})}{2}$$

Calculation of the variance (V_0) of T for H_0:

$$V_0(T) = \sum_{i=1}^{k-1} V_0(U_{i,i+1}) + 2\sum_{i=1}^{k-2} \operatorname{cov}(U_{i,i+1}, U_{i+1,i+2})$$

$$= \frac{1}{12}\sum_{i=1}^{k-1}(n_i)(n_{i+1})(n_i + n_{i+1} + 1) - \frac{1}{6}\sum_{i=1}^{k-2}(n_i)(n_{i+1})(n_{i+2})$$

The H_0 is rejected when $T \geq c$. The critical value ($\varnothing^{-1}$) of c depends on α, when

$$\frac{T - E_0(T)}{\sqrt{V_0(T)}} \geq \varnothing^{-1}(1-\alpha)$$

This can be rewritten in the form

$$\left(U_{i,i+1} - \frac{n_i(n_{i+1})}{2}\right) \Big/ \frac{\sqrt{(n_i)(n_{i+1})(n_i + n_{i+1} + 1)}}{12}$$

$$\geq \varnothing^{-1}\left(1 - \frac{\alpha}{k-1}\right)$$

The critical value of the test statistic is determined from $k-1$ samples, which is the desired number of ordered comparisons when following changes in gonadal mass. Once the ranks are determined for i and $i+1$ samples, the algorithm can be produced from the above using a computer worksheet.

Notations for calculating the test statistic

X_{ij} is the scaled gonadal mass measured on the jth individual on the ith period, N_i is the number of individuals in the ith period, and $R_{ij}^{(i+1)}$ is the rank associated with the jth individual of the ith period when all of the observations of the ith and $i+1$ period are ordered, such that $R_{ij} = X_{i,1}, X_{i,2}, \ldots, X_{i,n_i}$, and $X_{i+1,1}, X_{i+1,2}, \ldots, X_{i+1,n_i}$.

Microhomogenization of individual zooplankton species improves mercury and methylmercury determinations

Richard C. Back, Vanessa Visman, and Carl J. Watras

Abstract: A new method for processing zooplankton improves the accuracy and precision of mercury (Hg) and methylmercury (MeHg) determinations and permits the analysis of Hg species and ancillary variables, such as dry weight and C, N, and protein contents on subsamples of the same tissue homogenate using clean technique. In this study, 10–50 individual zooplankton were sorted from live samples, homogenized in 500 μL of low-Hg water, subsampled, and then digested for analysis without contamination. Detection limits ranged from 4 to 12 pg for Hg and from 0.8 to 4.3 pg for MeHg. Procedural blanks averaged 18.7 pg Hg and 5.3 pg MeHg. Yield from certified reference materials was consistently high on small masses of tissue homogenate (96% for Hg and 89% for MeHg), and recovery from spiked samples was good (99% for Hg and 92% for MeHg). We also present results for *Daphnia* and *Chaoborus* from Mud Lake, Wisconsin, processed in this manner. The new method improves our ability to determine the distribution of Hg species at lower levels of aquatic food webs and to investigate factors potentially regulating the bioaccumulation of Hg and MeHg in specific taxa.

Résumé : Une nouvelle méthode de traitement du zooplancton améliore l'exactitude et la précision des dosages du mercure (Hg) et du méthylmercure (MeHg), et permet l'analyse des espèces de Hg et des variables secondaires, comme le poids sec, le carbone, l'azote et les protéines dans des sous-échantillons du même homogénat de tissu obtenus par une méthode propre. Dans cette étude, 10–50 zooplanctontes ont été prélevés individuellement dans les échantillons vivants, homogénéisés dans 500 μL d'eau à faible teneur en Hg, subdivisés et soumis à la digestion en vue d'une analyse sans contamination. Les seuils de détection allaient de 4 à 12 ng pour Hg et de 0,8 à 4,3 pg pour MeHg. Les essais à blanc ont donné en moyenne 18,7 pg de Hg et 5,3 pg de MeHg. La récupération des produits témoins certifiés était régulièrement élevée sur les petites masses d'homogénat de tissus (96% pour Hg et 89% pour MeHg), et celle des échantillons enrichis était bonne (99% pour Hg et 92% pour MeHg). Nous présentons aussi les résultats obtenus sur des *Daphnia* et des *Chaoborus* provenant du lac Mud (Wisconsin) et traités de la même manière. La nouvelle méthode améliore notre capacité de déterminer la distribution des espèces de Hg aux niveaux inférieurs des réseaux trophiques aquatiques et d'examiner les facteurs qui peuvent régir la bioaccumulation de Hg et de MeHg chez certains taxons.

[Traduit par la Rédaction]

Introduction

The biomagnification of methylmercury (MeHg) in aquatic food webs substantiates the importance of diet as the predominant pathway for MeHg uptake. Supporting evidence comes from both theoretical (Rodgers 1994) and empirical (May et al. 1987; Lindqvist et al. 1991; Watras and Bloom 1992; Cabana et al. 1994) studies. Although the MeHg fraction increases with trophic position throughout aquatic food webs, the major bioconcentration step occurs at the level of bacteria and phytoplankton. Between water and phytoplankton, MeHg may increase by five orders of magnitude and then by factors of two to four times per trophic level thereafter (Meili 1991; Watras et al. 1994). These findings have increased interest in the speciation and concentration of mercury (Hg) in organisms of the lower food web. Because of their intermediate trophic position, zooplankton are thought to be a major conduit for MeHg to fish.

Previous studies of Hg in zooplankton have varied along a gradient of taxonomic specificity. Investigators have analyzed bulk assemblages comprising entire planktonic communities (e.g., Sorenson et al. 1990; Meili 1991). This composite approach would not be appropriate for fish communities, given the well-documented variability owing to

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size, age, and trophic position, and its utility with zooplankton remains questionable. Evidence of food chain amplification may be obscured in composite plankton samples (Knauer and Martin 1972). Alternative approaches include mechanical sorting into single species assemblages weighing a few grams (Rask et al. 1994) or hand sorting into smaller samples (ca. 1 mg) where individual zooplankton are selected one at a time to ensure that they are the same species, size, and ontogenetic stage (Watras and Bloom 1992; Back and Watras 1995).

While the single species approach is most appropriate for bioaccumulation studies of the lower food web, it is prone to contamination artifacts unless extraordinarily clean protocols are followed. To minimize sample handling and the potential for contamination, we previously recommended that the determination of the various constituents be made on different samples (Watras and Bloom 1992). Hg, MeHg, and dry weight were measured separately on different batches of 10–50 individuals of a given species. Because the digestion protocols for Hg and MeHg differ, there was an additional requirement for separate samples. As a result, the variance terms for our measurements were inflated, and subtle but significant differences between species and localities may have been missed. We now show that, with proper precautions, small numbers of zooplankton can be homogenized prior to digestion and subsamples taken for Hg, MeHg, and dry weight determinations from the same tissue homogenate.

Here we describe a modification of our previous method that permits multiple determinations from zooplankton homogenate without sacrificing taxonomic or ontogenetic specificity. The new technique involves microhomogenization in ultraclean tissue grinders. Our objective is to demonstrate that small samples can be homogenized without contamination and with quantifiable precision and accuracy, and to confirm our earlier measurements of MeHg in zooplankton. Sorting zooplankton of a particular species and homogenizing the sample allows parallel estimates of Hg, MeHg, and dry weight for the same material. This is particularly beneficial when the percent MeHg is of interest. Perhaps the most advantageous aspect of this approach is the ease with which aliquots for other determinations are obtained. We present, along with this method, results showing the dry weight and carbon, nitrogen, and protein contents of zooplankton samples. As more attention is directed towards Hg dynamics in the lower food web, correlations with such secondary variables could help to elucidate the mechanisms of biomagnification.

Materials and methods

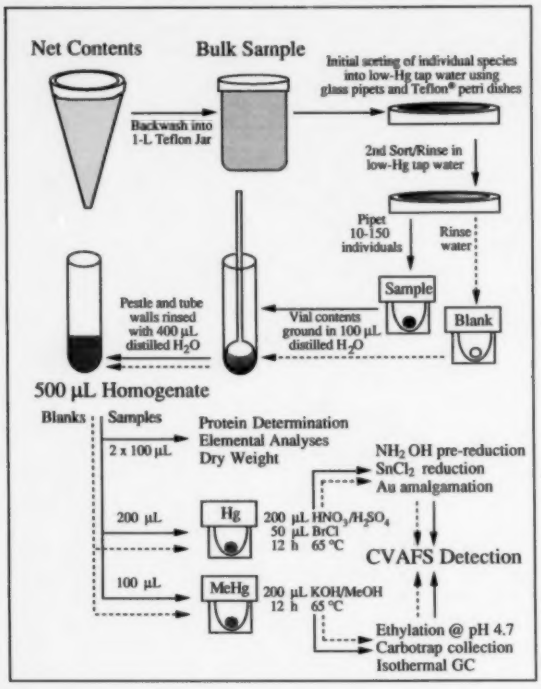
Two species of freshwater zooplankton and two certified reference materials were analyzed for Hg and MeHg. The zooplankton (*Daphnia pulex* and *Chaoborus americanus*) were collected from Mud Lake during the twilight hours of 6 June 1994. Mud Lake is a small (area 53 400 m^2; mean depth 5 m), dimictic, mesotrophic (average summer chloropyll *a* 8 μg/L), slightly colored (dissolved organic carbon 6 mg/L) lake located in the Northern Highlands Forest of Wisconsin, U.S.A. (46°N, 89°W). The certified reference materials (CRM) used were the dogfish liver DOLT-2 (National Research Council of Canada (NRCC), Ottawa, Ont.) and the marine copepod MA-A-1/TM (International Atomic Energy Agency, Monaco). DOLT-2 has certified values of 1.99 μg Hg/g dry weight (dw) and 0.673 μg MeHg/g dw. The copepods have a certified value of 280 ng Hg/g dw and a previously reported but not yet certified value for MeHg of 17–18 ng/g dw (Horvat 1991; Bloom 1992).

Bulk zooplankton was collected by replicate vertical hauls of a 20 cm diameter, 80-μm mesh, nonmetallic net (Aquatic Research Instruments, Seattle, Wash.). The sampling crew wore clean suits and shoulder-length plastic gloves and worked from a nonmetallic canoe. Net contents were backwashed using surface water into 1-L Teflon® jars. Sample jars were then double bagged and kept cool during transport back to the laboratory. Sample processing and Hg analyses were completed in a dedicated clean room. Bulk sample was sorted in a class-A laminar flow hood inside the clean room using Teflon and glass Petri dishes, and glass Pasteur pipets. All Teflon vials and glassware were cleaned by soaking for >8 h in hot (>100°C) concentrated HNO_3, followed by a low-Hg (<0.3 ng/L) water rinse and a 12-h soak in warm (65°C) 1% HCl. The glass grinder tubes and pestles were rinsed well with low-Hg water after use, and presoaked for 4 h in 30% KOH prior to acid cleaning.

The general protocol for homogenization and subsampling (Fig. 1) was modified when preparing reference materials as noted below. Samples were homogenized in 1-mL capacity borosilicate glass, microtissue grinders (Fisher Scientific). Typically 0.1–1 mg of material was loaded into the grinder tube along with 100 μL of low-Hg (~0.1 ng/L) distilled water. The sample was finely ground, and the pestle and the sides of the tube were rinsed with an additional 400 μL distilled water. For the zooplankton, four samples of 50 *Daphnia* (ca. 1 mg) or 10 *Chaoborus* (ca. 4 mg) were ground in separate tubes. From each grinder, 200 μL was collected for Hg, 100 μL for MeHg, 100 μL for dry weight, and 75 μL for protein analysis. Subsamples for Hg and MeHg analyses were transferred to 3-mL Teflon vials (Savillex Corp., Minnetonka, Minn.) and frozen until analysed. Subsamples for dry weight determinations were dispensed into preweighed Sn boats, dried overnight at 65°C, and stored in a desiccator at room temperature. Subsamples for protein analysis were collected in 2-mL Eppendorf® centrifuge tubes and frozen. A similar scheme was followed for the CRM except that four replicate subsamples for Hg and MeHg were taken from each of three grinder tubes. To one of each of these replicates we added either 0.095 ng Hg or 0.018 ng MeHg to assess spike recovery during digestion and analysis.

Sample homogenate was digested overnight at 65°C, using 200 μL of HNO_3–H_2SO_4 (5:2 v/v) and 50 μL BrCl (5% v/v) for the Hg analysis or 200 μL KOH–methanol (1:4 w/v) for the MeHg analysis. Reagents were added to thawed sample in the Teflon vials, which were recapped and sealed in temperature-resistant plastic bags before being placed in the oven. The Hg digestates were prereduced by the addition of 50 μL NH_2OH–HCl (30% w/v) 20 min prior to analysis. Hg was determined by cold-vapor atomic fluorescence following the protocol of Bloom and

Fig. 1. General protocol for the homogenization of zooplankton and subsampling for Hg, MeHg, dry weights, and other ancillary variables. Broken line tracks procedural blanks.



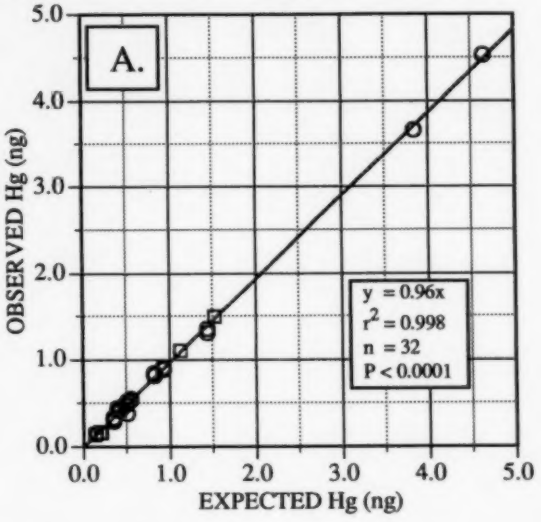
Fitzgerald (1988). Determination of MeHg followed aqueous phase ethylation, carbotrap collection, separation by isothermal gas chromatography, and cold-vapor atomic fluorescence detection using a modification of the method of Bloom (1989). The pH of the MeHg digestates was adjusted to pH ~4.7 in the sparging vessel by the addition of 100 µL of an acetate buffer (2 M sodium acetate in 2 M acetic acid) prior to the addition of ethylating agent. Output from the fluorescence detector was quantified with an HP3396 series II integrator. Peak areas were used in all calculations. Hg (nanograms) and MeHg (as nanograms Hg) were calculated from aqueous standards; nonmethyl Hg (inorganic Hg) was estimated as the difference between paired Hg and MeHg values.

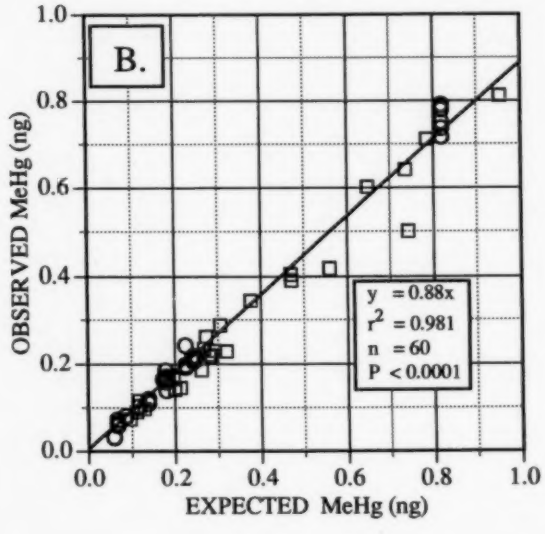
Dry weights were measured to the nearest 0.1 µg on a Cahn C-31 electrobalance. Carbon and nitrogen contents were measured on the dry weight samples using a Carlo Erba elemental analyzer with acetanilide as the standard. Protein analyses followed the Bradford (1976) modification of the Coomassie Brilliant Blue G-250 procedure with bovine serum albumin as the standard.

Results

The digestion and extraction protocols were equally efficient over a wide range of sample Hg masses whether or not CRM was ground in the homogenizer tubes. The yield of Hg averaged 96% from DOLT-2 and MA-A-1/TM over

Fig. 2. Yield of Hg and MeHg from different amounts of certified reference material with (circles) and without (squares) grinding in the microhomogenizer tubes. (A) Observed and expected Hg (ng) from the DOLT-2 (dogfish liver) and MA-A-1/TM (marine copepod) certified reference materials. (B) Observed and expected MeHg (as ng Hg) from the DOLT-2 certified reference material.





the expected range of 0.15–4.65 ng Hg (Fig. 2A). The yield of MeHg from the DOLT-2 (Fig. 2B) was lower and more variable but averaged 88% over the expected range of 0.06–0.95 ng MeHg.

The agreement among replicate samples of certified reference material demonstrated that sample homogenization by grinding can yield accurate results with a reasonable degree of precision during both Hg and MeHg analysis (Table 1). We found satisfactory reproducibility of Hg estimates from

Table 1. QA/QC (quality assurance – quality control) analyses during the homogenization procedure: yield of Hg and MeHg from certified reference materials.

	Hg					MeHg				
	Mean (ng/g)	95% CI	SD	CV (%)	*n*	Mean (ng/g)	95% CI	SD	CV (%)	*n*
				DOLT-2						
	2014	86	35	2	3	641	130	52	8	3
	1960	39	16	1	3	586	164	66	11	3
	1969	69	28	1	3	629	208	84	13	3
Mean	1981	71	29	2	3	619	72	29	5	3
Certified	1990	100	—	—	—	673	53	—	—	—
				MA-A-1/TM						
	245	30	12	5	3	31	3	1	4	3
	210	24	10	5	3	20	8	3	15	3
	234	21	9	4	3	6	1	1	9	3
Mean	230	45	18	8	3	19	31	13	66	3
Certified	280	10	—	—	—	17[a]	—[a]	1[a]	—[a]	3[a]
						18[b]	—[b]	2[b]	—[b]	3[b]

Note: Shown are the means of triplicates from each of three grinder tubes, 95% confidence intervals, standard deviations, and coefficients of variation. Also shown are statistics for the mean of the three grinder tubes, and available statistics on the certified materials.

[a]Previously reported estimates on uncertified material, from Bloom (1992).

[b]Previously reported estimates on uncertified material, from Horvat (1991).

successive pipettings from a given grinder tube. The coefficient of variation of subsamples for DOLT-2 homogenate ranged from 1 to 2%, and from 4 to 5% for the MA-A-1/TM homogenate. Results of MeHg analyses on CRM replicates were more variable. The coefficient of variation for subsamples of DOLT-2 ranged from 8 to 13%, and for the MA-A-1/TM it ranged from 4 to 15%.

Recovery of aqueous Hg and MeHg spikes averaged 100 and 90% for DOLT-2, and 98 and 94% for MA-A-1/TM (Table 2). Procedural blanks (distilled water taken through the homogenization and digestion steps) during Hg analyses were not significantly higher than reagent blanks (one-tailed *t* test, $P = 0.3$), indicating that Hg contamination during this procedure was negligible. Procedural blanks averaged 18.7 pg during Hg analyses, and 5.3 pg during MeHg analyses (Table 2). Detection limits, here defined as three times the standard deviation of procedural blanks, during these analyses averaged 12.4 pg Hg and 2.8 pg MeHg (Table 2).

Dry weights of the four homogenized samples of 50 *D. pulex* ranged from 168 to 260 μg/100 μL. The four samples of 10 homogenized *C. americanus* ranged from 748 to 946 μg/100 μL. *Daphnia* samples ranged from 409 to 524 mg protein/g dw and averaged 37% carbon and 8% nitrogen by dry weight. *Chaoborus* samples averaged 39% carbon and 10% nitrogen by dry weight and two of the samples yielded measurements of 416 and 408 mg protein/g dw.

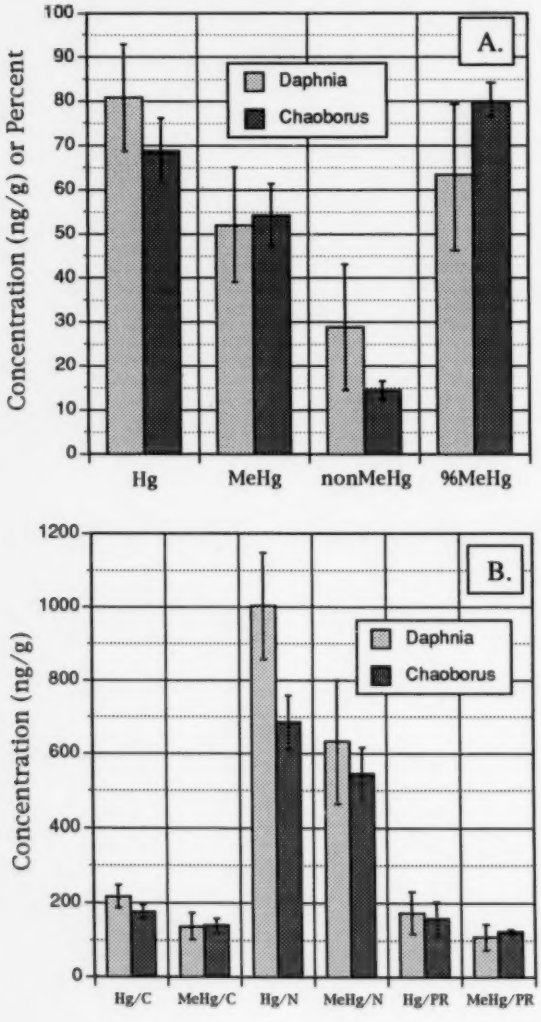
Hg concentrations of the zooplankton homogenate (Fig. 3A), corrected for CRM yields, ranged from 71 to 89 ng Hg/g (80 ± 7.6; mean ± SD) and from 41 to 61 ng MeHg/g (51 ± 8.2) for the *Daphnia* Hg concentrations in *Chaoborus* ranged from 63 to 74 ng Hg/g (69 ± 4.7) and

Table 2. QA/QC (quality assurance – quality control) analyses during the homogenization procedure: percent recovery of aqueous spike added to CRM, procedural blanks, and daily detection limits during the period of analyses.

	Hg			MeHg		
	Mean	Range	*n*	Mean	Range	*n*
Spike recovery (%)						
DOLT-2	100	99–101	3	90	76–101	3
MA-A-1/Tm	98	94–100	3	94	90–97	3
Blanks (pg)	18.7	12–26	21	5.3	0.6–1.0	35
Detection limits (pg)	8.0	4–12	12	2.4	0.8–4.3	11

from 50 to 60 ng MeHg/g (55 ± 4.4). While the *Daphnia* had slightly higher Hg concentrations than *Chaoborus* ($P < 0.05$ in a one-tailed *t* test), the two taxa had similar MeHg concentrations ($P = 0.66$ in a two-tailed *t* test). The portion of Hg determined to be MeHg in paired samples was therefore higher in the predatory *Chaoborus* than in the herbivorous *Daphnia*. A one-tailed, unpaired *t* test showed that this difference was significant ($P < 0.01$) for the arcsine-transformed percentages. The mean MeHg percentages (back-transformed) were 63% for *Daphnia* and 80% for *Chaoborus*. The most significant difference between the *Daphnia* and *Chaoborus* samples was in the nonmethyl Hg fraction: 29 ng/g in *Daphnia*, and 15 ng/g in *Chaoborus* ($P = 0.0176$). Similarity between *Daphnia* and *Chaoborus* was also evident when mercury concentrations were normalized to the carbon, nitrogen, and protein contents of the samples (Fig. 3B). Nitrogen-corrected Hg (nanograms

Fig. 3. Mercury content and speciation in zooplankton samples from Mud Lake, Wisconsin. Each column represents the mean of four samples, the error bars are ± 95% confidence intervals, and the data are yield and blank corrected. (A) Total, methyl, and nonmethyl mercury data normalized to dry weight of sample, and the percent MeHg of Hg. The same numerical scale applies to both the concentration and the percentage. (B) Data normalized to carbon (C), nitrogen (N), and protein (PR) content of samples.



Hg per gram N), however, did show higher concentrations in *Daphnia*.

Discussion

The results for zooplankton from Mud Lake, homogenized and subsampled following the new protocol, were encouraging. The variability among replicate determinations of Hg and MeHg concentrations (dry weight basis) in *Daphnia* and *Chaoborus* was low, and our ability to detect subtle but potentially important differences between these taxa was improved. The variability between dry weight estimates on groups of individuals (CV = 21% for *Daphnia* sp. and CV = 10% for *Chaoborus*) reflects the natural variability between individuals, even when sorted. This variability demonstrates the error that may arise from using such samples for separate Hg, MeHg, and dry weight measurements. For example, if we had used one of the *Daphnia* samples to estimate dry weights for all other samples, our estimated concentrations could have ranged from 51 to 132 ng Hg/g and from 24 to 87 ng MeHg/g. Because we had dry weight estimates for each of the *Daphnia* samples, our Hg and MeHg concentrations are inherently more precise, ranging from 71 to 89 ng Hg/g and from 41 to 61 ng MeHg/g. Concentrations in *Chaoborus* ranged from 63 to 74 ng Hg/g and from 50 to 60 ng MeHg/g. If we had not had a dry weight estimate for each sample, however, the estimated concentrations in *Chaoborus* could have ranged from 50 to 92 ng Hg/g and from 40 to 74 ng MeHg/g. This comparison of new and old protocols indicates an improvement in precision of three to four times with the new method.

Such a comparison of methods is more dramatic when one considers the estimation of the portion of Hg that is in the form of MeHg (percent MeHg). The percent MeHg is of interest because it is the methyl fraction that increases with trophic level. By homogenizing the sample prior to analysis, and subsampling for both Hg and MeHg analyses, we can obtain more reliable estimates of percent MeHg. In our paired samples, this value ranged from 57 to 78% for *Daphnia*, and from 77 to 82% for *Chaoborus*. If the samples had not been paired, we could have observed ranges of 46–86% and 68–95%, respectively.

We emphasize that our sample size for these determinations was low ($n = 4$) because the purpose was to test the method rather than to test specific hypotheses about bioaccumulation. Nevertheless, the data presented (Fig. 3) support our previous observations of Hg in the lower food web. Back and Watras (1995) compared bioconcentration in *Daphnia* spp. and *Chaoborus*, among other species, in 12 Wisconsin lakes and reported patterns different from those expected for a herbivore–predator relationship. Although there were wide ranges for both genera, *Daphnia* tended to have a bioconcentration value greater than or equal to that of *Chaoborus*. In samples specifically from Mud Lake, sampled in October 1993, we observed Hg concentrations in *D. pulex* of 82 ng/g and in *C. americanus* of 60 ng/g. Our present data confirm these observations. However, the paired Hg and MeHg data of the present study do indicate a higher proportion of MeHg in the predator (*C. americanus*). This result is consistent with current hypotheses about MeHg enrichment up the food chain (May et al. 1987; Watras et al. 1994).

We conclude that microhomogenization can improve the precision of Hg and MeHg determinations in small masses of biological samples without introducing significant contamination. Since multiple determinations can now be performed on the same homogenate, it should be possible to track the distribution of mercury species in relation to

other biotic constituents, such as C, N, S, and protein, at the lower levels of aquatic food webs. This method will allow us to test more rigorously hypotheses about factors regulating the bioaccumulation of mercury.

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The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator

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Abstract: Differences in selection regimes between hatchery and natural environments and environmental stimuli, among other factors, have the potential to cause differences in predator avoidance ability between hatchery and wild steelhead trout fry (*Oncorhynchus mykiss*). In two separate laboratory experiments, fry raised from eggs of wild Quinault River steelhead trout survived predation by prickly sculpin (*Cottus asper*) significantly better than size-matched offspring of a locally derived hatchery population, which were reared under similar conditions. Wild fry also survived predation better than hatchery fry in natural stream enclosures over a 3-day test period. Experience, in the form of 50-min visual exposure to sculpin predation on "sacrificial" steelhead trout, improved the ability of fry from both populations to avoid predation by sculpin. Wild-experienced fry were eaten in the fewest number of trials followed by wild-naive, hatchery-experienced, and hatchery-naive fry. The results of this study are consistent with the hypothesis that innate predator avoidance ability has been negatively altered through domestication and that attempts to condition hatchery-reared steelhead to avoid predators may be limited for domesticated populations.

Résumé : Les différences dans le régime de sélection selon l'origine artificielle et naturelle, et les stimuli environnementaux, entre autres facteurs, peuvent causer des différences dans la capacité d'éviter les prédateurs entre des alevins d'élevage et des alevins sauvages de saumon arc-en-ciel (*Oncorhynchus mykiss*). Dans deux expériences de laboratoire distinctes, des alevins provenant d'oeufs de saumons arc-en-ciel sauvages de la rivière Quinault ont nettement mieux survécu à la prédation par le chabot piquant (*Cottus asper*) que la progéniture de taille comparable d'une population d'élevage provenant de la région et élevée dans des conditions similaires. Les alevins sauvages ont aussi mieux survécu à la prédation que les alevins d'élevage en enclos, dans un ruisseau naturel, pendant un essai de 3 jours. L'expérience, par exposition visuelle de 50 min à la prédation par les chabots de saumons arc-en-ciel sacrifiés, a amélioré chez les alevins des deux populations la capacité d'échapper à la prédation par les chabots. Ce sont les alevins sauvages initiés qui ont été mangés dans le plus petit nombre de cas, suivis par les alevins sauvages novices, puis les alevins d'élevage initiés, puis les alevins d'élevage novices. Les résultats de l'étude confortent l'hypothèse selon laquelle l'aptitude innée à l'évitement des prédateurs est négativement affectée par la domestication, et les tentatives de conditionnement des saumons arc-en-ciel d'élevage pour qu'ils évitent les prédateurs semblent n'avoir que des effets limités dans les populations domestiquées.

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Introduction

Substantial research has demonstrated that domestication (Utter et al. 1993) of anadromous salmonid populations results in phenotypic divergence from wild founder populations for a host of biological characters (see Leider et al. 1990). For the case of steelhead trout (*Oncorhynchus mykiss*) in the Pacific Northwest, the phenotypic differences

attributable to domestication may reduce the reproductive success of hatchery populations. Empirical evidence exists that naturally produced progeny of wild steelhead populations survive better than offspring of locally derived domesticated populations during egg-to-fry, fry-to-smolt, and smolt-to-adult stages (Reisenbichler and McIntyre 1977; Chilcote et al. 1986; Leider et al. 1990).

In addition to evidence that environmental rearing conditions can strongly influence various behavioral characteristics of juvenile salmonids (Fenderson and Carpenter 1971; Dickson and MacCrimmon 1982; Bachman 1984), recent studies also demonstrate that several generations of hatchery propagation may alter innate behavioral characteristics such as aggression (Swain and Riddell 1990) and antipredation tactics (Johnsson and Abrahams 1991). These studies suggest there is a genetic basis for some behavioral differences between hatchery and wild fish, which may reduce the fitness of hatchery fish in natural environments. A direct link, however, between behavioral characteristics (e.g., antipredation responses) of hatchery fish and their reduced survival during juvenile stages has yet to be demonstrated.

Predation can be a major source of mortality for juvenile salmonids (Ruggerone and Rogers 1984; Fresh and Schroder 1987). In freshwater lakes and streams, sculpins (*Cottus* sp.) have been observed to prey heavily on age 0 salmonid fry (Ricker 1941; Hunter 1959). Patten (1975) suggested that because of certain innate behaviors such as vertical position in the water column and microhabitat use, steelhead fry were more vulnerable to predation by sculpins than any of the five species of North American Pacific salmon (*Oncorhynchus* spp.). Predation by sculpins on naturally produced steelhead fry in streams may induce selective pressures favoring antipredation responses. Hatchery-produced steelhead are too large to be vulnerable to sculpin when released as age 1 smolts. Natural selection against risk-averse phenotypes in streams may be relaxed on fry reared in predator-free hatcheries, which may increase the proportion of risk-taking individuals in hatchery populations over generations. If the differences in selection regimes between natural and hatchery environments are strong, innate antipredation responses of hatchery steelhead populations may differ from those of wild populations after a relatively few generations of culture.

In conjunction with possible genetic changes that may occur through domestication, typical hatchery-rearing environments possibly obscure the development of antipredation responses within an individual's lifetime because hatcheries lack sensory stimuli associated with predation. Predator avoidance ability of juvenile salmonids can significantly improve after prior exposure to predators (Thompson 1966; Ginetz and Larkin 1976; Patten 1977; Olla and Davis 1989). It is unknown, however, whether or not such experiences can fully compensate for potential deficits in predator avoidance ability that may exist for hatchery fry. For cases in which fry planting might be a reasonable supplementation option (see Steward and Bjornn 1990), it would be especially important to understand the combined influence of domestication and experience on the ability of steelhead fry to avoid naturally occurring freshwater predators.

The primary objective of this study was to assess the relative predator avoidance ability of F_1 steelhead fry from wild and hatchery populations of common ancestry. The second objective was to investigate the interactive effects of wild and hatchery ancestry and experience on the relative ability of steelhead fry to avoid predation by sculpins.

Materials and methods

Study populations

Eyed eggs from seven pairs (one to one matings) of wild Quinault River steelhead were collected at the Quinault Department of Natural Resources pen rearing facility on Lake Quinault, Washington, located approximately 120 km west of Seattle. Wild Quinault River steelhead were identified by the absence of any fin damage common to hatchery steelhead. Subsequent scale analysis confirmed that these steelhead had narrowly spaced freshwater circuli and migrated to sea after 2 years in fresh water, hence were produced naturally, not in a hatchery (cf. Leider et al. 1990).

Eyed eggs were also obtained from 10 pairs (one to one matings) of hatchery steelhead spawned at the Quinault National Fish Hatchery on Cook Creek, a tributary to the lower Quinault River. The Cook Creek population was derived solely from wild adult steelhead captured in the main stem of the lower Quinault River from 1972 to 1974. There is no evidence that any steelhead have ever been introduced to the Quinault National Fish Hatchery or Quinault River system from any other river system. Cook Creek spawners in 1994 were at least one generation removed from their wild ancestors, and given the ages of Cook Creek steelhead (ranging from ages 1.2 to 1.4), may have been as many as four to seven generations removed. Scale analysis showed that Cook Creek brood fish migrated to sea after 1 year of freshwater residence and had wide, uniform spacing of freshwater circuli, indicating that these fish were reared in the hatchery as juveniles.

Eyed eggs were transported to the University of Washington's Big Beef Creek Research Facility, Kitsap County, Washington, and incubated in Heath trays. At emergence, Cook Creek fry (placed in rearing tanks from May 26 to May 27, 1994) and wild Quinault fry (placed in rearing tanks from May 31 to June 1, 1994) were reared in separate, square rearing tanks (1.8 × 1.8 m wide by 0.5 m deep), supplied with 10–10.5°C well water at a rate of 23 L/min. Fry were stocked in the tanks at an initial stocking density of 2469 fish/m^3. Fry from both populations were scatter fed a semimoist crumble four times daily at a ration of 2% of biomass per day until they were removed for experiments.

Prickly sculpin were collected in minnow traps from Big Beef Creek, placed in a 400-L tank, and maintained on a daily ration of frozen thawed steelhead eggs for approximately 3 weeks before experiments were conducted. Sculpin ranged in size from 105 to 125 mm total length.

Predation on hatchery and wild fry in flume chambers

Apparatus

The first experiment was conducted to test the null hypothesis of no difference in the relative ability of Cook Creek

and Quinault steelhead fry to avoid predation by prickly sculpin. The experiment was conducted in two 10.0 × 1.2 m wide wooden flumes. The two flumes were divided in half longitudinally, creating four channels, each with 10 Plexiglas viewing windows measuring 41.0 cm wide by 30.5 cm high. During daylight hours an intensity of 600-lx illumination at the water surface was provided by five pairs of wide-spectrum 40-W fluorescent bulbs over each flume. The flumes were covered with black plastic sheets so that minimal light was able to escape to the observer area. This was sufficient to eliminate disturbance to the fish during observation. Light intensity during nighttime was reduced to 30 lx and was provided by fluorescent bulbs approximately 3.0 m from the flumes penetrating through the observation windows.

Trials were conducted from June 23 to July 1, 1994, in 12 nonadjacent chambers (64 cm long × 61 cm wide × 27 cm deep) situated in the flume channels. A total of 48 trials was conducted in four sets of 12 (i.e., the number of available chambers). The upstream and downstream barriers of each chamber were constructed of fine mesh nylon netting. A layer of gravel (0.5–2.0 cm in diameter) covered the bottom of each chamber to a depth of 5.0 cm. Water was recirculated through the flumes, creating a flow of 8.4 L/s through each chamber.

Procedures

A sample of fry from each population was removed from the rearing tanks without conscious selection, held separately, and anesthetized with tricane methanesulfonate (MS-222). For each trial, three fry were then specifically selected from each population such that the range in fork length within a population for a given trial was not more than 1.5 mm, and each individual from one population had a counterpart from the other population that did not differ by more than 0.5 mm (measurement error). The mean fork length of hatchery and wild fry in the rearing tanks did not differ at the time this experiment was conducted. The size-matching procedure was followed to minimize the confounding effects of size-selective predation (Parker 1971; Ware 1972; Patten 1977).

Adipose fins were clipped on all individuals from one or the other population in each trial, and this procedure was rotated between populations such that each population received fin clips in half of the 48 trials. There was no statistically significant effect of fin clipping on susceptibility to predation between any of the groups so treated in this experiment or in either of the following two experiments.

Each group of six fry was placed into a chamber and left to acclimate for 1.5 h before one sculpin was introduced into the downstream end of the chamber. The sculpin generally lay motionless and invariably took at least 2 h to acclimate and to begin stalking steelhead fry. Each trial was started at 16:00 on day 1. The chambers were then checked for survivors at 10:00 on day 2 (18-h exposure) for all 48 trials. The data (number of fry eaten from each population) were analyzed by a Wilcoxon signed-ranks test (Siegel and Castellan 1988) with the Z statistic corrected for ties. The first 15 trials were terminated after 18-h exposure of fry to sculpin, but the surviving fry were left exposed to the sculpin in 33 of the 48 trials for an additional 24 h (total of 42-h exposure) to observe whether differences in relative vulnerability to predation persisted after more prolonged exposure of fry from both populations to the sculpin.

Population and visual experience effects

Apparatus

A second experiment was conducted from June 28 to July 7, 1994, to test the null hypothesis that population of origin and prior visual experience have no effect on the ability of steelhead fry to avoid predation by prickly sculpin. Fry from both populations were presented with visual stimuli (i.e., experience) associated with predation by sculpin without actually being vulnerable, thereby eliminating the possibility that less fit individuals would be culled from the subject pool (Olla and Davis 1989).

Fry received experience in a Plexiglas aquarium (58 cm wide × 61 cm long × 40 cm deep) situated in one of the flume channels so it could be observed through one of the observation windows. The upstream and downstream ends of the aquarium were constructed of 8-mm square mesh galvanized screen to allow a unidirectional, 27 cm deep, laminar flow of water through the entire aquarium. A clear Plexiglas divider bisected the aquarium longitudinally from top to bottom. Six prickly sculpin were placed into the portion of the conditioning aquarium furthest from the observation window and were allowed to acclimate for at least 24 h prior to a conditioning session. The bottom of the aquarium was covered with approximately 5 cm of gravel 0.5–2.0 cm in diameter.

The control aquarium had the same physical dimensions as the conditioning aquarium but contained no sculpin. Fry in the control aquarium (i.e., naive fry) were handled in the same manner as experienced fry.

Subsequently, predator avoidance challenges (described below) were conducted in 19-L aquaria situated on wooden frames inside the flumes so that each aquarium could be seen through an observation window. Water was recirculated through the flumes at a depth of 27 cm (just below the tops of the aquaria) to provide clear visibility of the aquaria. Each aquarium received 0.5 L/min of 10.5°C well water sprayed through a 3-mm mesh screen covering the top of each aquarium. Gravel substrate was added to the aquaria at a depth of 3–4 cm.

Procedures

Subjects from each population were removed without conscious selection from their respective rearing tanks. Twelve individuals from each population were then selected within a narrow size range (40 ± 0.5 mm) to minimize size-predation confounding effects, and half were adipose clipped to distinguish between individuals of the two populations. Six individuals from each population were then simultaneously placed into either the conditioning aquarium or the control aquarium to create four combinations of population and experience as follows: Cook Creek (hatchery), naive (CN); Cook Creek, experienced (CE); Quinault (wild), naive (QN); and Quinault, experienced (QE).

Each conditioning session (two sessions/day) was conducted as follows. Twelve steelhead fry, including 6 from

each population, were introduced into the near portion of the conditioning aquarium, separated from the sculpin by the Plexiglas divider. Quinault and Cook Creek fry were always conditioned together to ensure that fry from both populations received equal visual exposure to predation. The number of attacks by sculpin through the glass was recorded for 15 min. After 15 min, six sacrificial steelhead were added to the far side of the aquarium where they could be eaten by the sculpin, which could be observed by the 12 subjects. For the next 15 min the number of attacks by sculpin through the glass as well as failed and successful attacks on the six sacrificial steelhead were recorded. Subsequently, six additional sacrificial fry were placed in the far side of the aquarium and the same data were recorded for an additional 20 min (total conditioning time 50 min).

Solid Plexiglas flow barriers were placed at the upstream and downstream ends of the control and conditioning aquaria after the 50-min conditioning period, and the fish were administered a mild dose of MS-222 to limit their escape response while being netted. The subjects were removed from the two aquaria and placed into one of four 6.0-L holding containers depending on their treatment designation (CN, CE, QN, or QE). The entire conditioning process was repeated as necessary to conduct all of the predator avoidance trials described below.

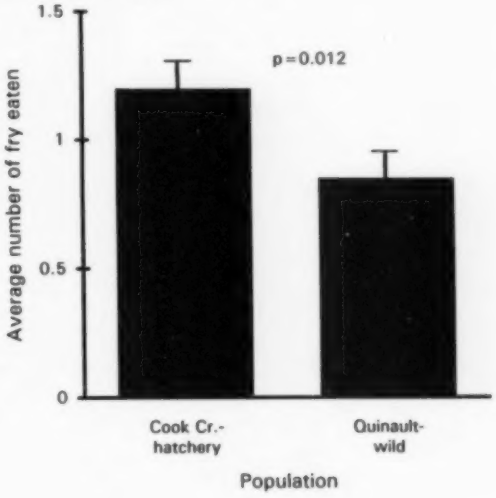
Immediately after conditioning, one adipose-clipped fry was paired with one nonclipped fry and both fish were challenged to avoid predation by placing them into one of the 19-L aquaria with the sculpin, but the pair of steelhead were separated from the sculpin by a solid opaque plastic barrier for a 24-h acclimation period. After acclimation the dividers were removed and the tanks were observed at intervals of not more than 10 min until the first steelhead fry in each aquarium was eaten. The population and experience of the captured fish were identified by the presence or absence of an adipose clip on the surviving fish. The surviving fish was then removed. Trials were conducted until there were 10 cases where one fish was eaten within 3 h ($N = 60$) for each of the following six paired combinations: CE versus CN, CE versus QE, CE versus QN, CN versus QE, CN versus QN, and QE versus QN; thus, the experiment was balanced. Data were analyzed by a chi-square contingency table where the two rows were the outcomes (eaten or escaped) and the four columns were the treatments (QN, QE, CN, CE). Under the null hypothesis, half of the fry from each treatment would be eaten and half would escape (i.e., the expected frequency for each cell was 15). Significant deviations from expected frequencies would suggest that the four treatment groups were not equal in their ability to avoid predation, relative to fry from the other treatments.

Predation in a seminatural stream

A third experiment was conducted from July 16 to July 19, 1994, to test the null hypothesis that there was no difference in the relative ability of Cook Creek and Quinault steelhead fry to avoid predation by prickly sculpin in a seminatural stream environment. The experiment was conducted in a 34.2 m long × 6.2 m wide section of a side channel of Big Beef Creek. The stream channel provided natural vegetation, photoperiod, food, gravel substrate, and temperature fluctuation (Spalding et al. 1995). The channel was bisected along its entire length by a vertical plywood divider and divided cross-sectionally into seven distinct habitat units (4.9 m long × 3.1 m wide) on each side of the longitudinal divider. The 14 units were alternating pool (mean velocity 4 cm/s, mean depth 19.8 cm) and riffle (mean velocity 11.5 cm/s, mean depth 10.1 cm) habitats, separated from each other by wood-framed, 8-mm mesh galvanized screens, which completely restricted both steelhead fry and sculpin from moving between units.

Thirteen fry from each population were introduced into each habitat unit followed by five sculpin, 2 h later. The fish were again selected so there was a one to one correspondence in length–frequency distributions between the two populations within each unit. Adipose clips were utilized to distinguish members of the two populations. The units were then sampled 3 days later with beach seines to enumerate survivors. At least six seine hauls were made through each unit, but hauls were continued until there were three consecutive hauls in which no fish were caught. The numbers of survivors per habitat unit from each population were compared by a Wilcoxon signed-ranks, Z statistic corrected for ties, and the number of survivors in pools and riffles was analyzed by a Mann–Whitney U test.

Fig. 1. The average number of fry (±SE) from each population (matched for size) eaten after 18 h of exposure to a single sculpin.

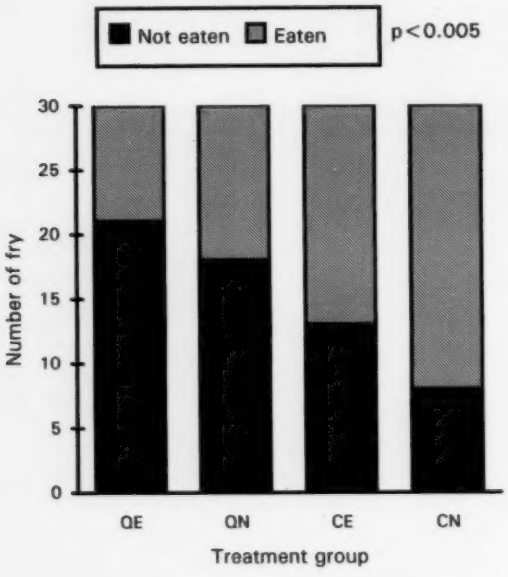


Results

Predation on hatchery and wild fry in flume chambers

Significantly more Cook Creek (hatchery) steelhead fry were eaten than Quinault (wild) fry after 18-h ($Z = -2.53$, $N = 48$, $p = 0.012$) exposure to a single sculpin in the flume chambers. Sculpin ate an average of 1.2 Cook Creek fry and 0.85 Quinault fry after 18 h (Fig. 1). For the 33 trials in which the surviving steelhead fry were left in the

Fig. 2. The number of fry that were eaten and not eaten in round-robin trials for each treatment group. Q, Quinault (wild) population; C, Cook Creek (hatchery) population; E, experienced; N, naive. Each treatment group was involved in 10 paired contests with each other group ($N = 60$).



enclosures with the sculpin for an additional 24 h, an average of 0.60 Cook Creek and 0.45 Quinault fry were eaten by sculpin during the 24-h period, even though more wild Quinault fry than Cook Creek fry were available to sculpin after 18 h. The average number of fry (both populations combined) eaten by individual sculpin increased from 2.08 (35%) after 18 h to 3.16 (53%) after 42 h.

Population and experience effects

During the 50-min conditioning periods, steelhead fry from both populations were equally subjected to an average of 4.4 attacks by sculpin attempting to capture the test fry through the glass divider in the conditioning aquarium. Sculpin also made an average of 6.0 failed attacks and 2.2 successful attacks on sacrificial steelhead.

Quinault experienced (QE) fry were captured least often, followed by QN, CE, and CN fry (Fig. 2). The differences in the relative abilities of the four treatment groups to survive predation were significant ($\chi^2 = 13.07$, df = 3, $p < 0.005$). An a posteriori analysis of the adjusted standardized residuals in each cell revealed that the significance of the overall test was primarily accounted for by deviations from expected frequencies under the null hypothesis for CN and QE fry. Cook Creek naive fish were eaten in 22 out of 30 trials, which was more often than expected ($Z = 3.0$, $p = 0.003$), while only nine QE fish were eaten, which was fewer than expected ($Z = 2.5$, $p = 0.012$). Thus, both population and experience factors affected relative vulnerability to predation.

Table 1. Mean number of steelhead fry from each population surviving (out of 13) after 3 days in pool and riffle units in the Big Beef Creek side channel.

	Pools		Riffles	
Population	Mean ± SE	*N*	Mean ± SE	*N*
Quinault, wild	10.4±0.9	7	9.0±0.9	7
Cook creek, hatchery	8.7±0.6	7	6.4±0.8	7

Predation in a seminatural stream

Of the 26 fish initially placed in each unit, an average of 7.6 (58%) Cook Creek and 9.7 (75%) Quinault fry survived after 3 days, which was a significant difference between the two populations ($Z = -2.24$, $N = 14$, $p = 0.025$). There was also a significant difference between pools and riffles in the total number (both populations combined) of fry recovered ($Z = -2.26$, $N = 7$, $p = 0.023$). An average of 15.5 fry remained in riffles and 19.1 fry remained in pools after 3 days. Fry from both populations survived better in pools than in riffles (Table 1).

Seven of the 28 sculpin recovered at the same time fry were removed on July 19 contained an average of 1.5 fry in their stomachs. Some fry may have been eaten by avian predators (e.g., belted kingfishers, *Ceryle alcyon*, green-backed herons, *Butorides striatus*, and blue herons, *Ardea herodias*), but avian predation has been shown to be minimal in the Big Beef Creek side channel in several previous studies (Spalding et al. 1995; Quinn et al. 1995; B.A. Berejikian, unpublished data).

Discussion

Poor fry-to-smolt survival of domesticated steelhead such as that observed by Chilcote et al. (1986) and Reisenbichler and McIntyre (1977) may be partly a result of inadequate predator avoidance ability by offspring of domesticated steelhead fry. My results clearly demonstrate that the relative ability of hatchery steelhead fry to avoid a benthic freshwater predator was significantly less than that of wild fry in two separate laboratory experiments. The relevance of these results to a natural situation was demonstrated by the third experiment, in which wild fry survived better than hatchery steelhead in 14.9-m^2 natural stream enclosures over a 3-day test period. Fry from the two populations were reared under identical conditions prior to the experiments, which suggests that the differences in predator avoidance ability were genetically determined. Johnsson and Abrahams (1991) found that domesticated rainbow trout × wild steelhead hybrids were more willing to forage in the presence of a predator than wild steelhead fry, suggesting that domestication may increase risk-taking behavior and increase susceptibility to predation. When individually challenged to survive 15 s of exposure to a piscine predator, however, domestic × wild hybrids were no better able to survive predation than wild steelhead. Increased risk-taking behavior without a concomitant increase in predator avoidance ability may increase vulnerability to predation.

It is possible that the comparison of hatchery and wild populations in my study may have been confounded by the existence of genetically distinct subpopulations of winter steelhead within the Quinault River system. Wild spawners were collected for this study in Lake Quinault and were probably destined to spawn in small tributaries to Lake Quinault or in the upper Quinault River system. Initial brood stock for the Quinault National Fish Hatchery (i.e., the Cook Creek population) was collected below Lake Quinault so their eventual spawning destination may have been the lower river, Lake Quinault tributaries, or the upper river. Reisenbichler and Phelps (1989) suggested that Lake Quinault may serve as a partial barrier to gene flow between the upper and lower river that allowed the upper Quinault population to genetically differentiate from the lower Quinault and other populations. However, Reisenbichler and Phelps (1989) also concluded that genetic differences detected by electrophoresis between Cook Creek steelhead and wild Quinault steelhead and other Washington coastal populations were an artifact of the hatchery program. I believe that the wild brood stock collected in Lake Quinault for this study were the closest to the donor wild population available and provide for a valid comparison between the Cook Creek hatchery and Quinault wild populations.

Theoretically, selection regimes for cultured and wild populations inevitably differ, even when the hatchery environment represents only a portion (usually egg to smolt) of the life cycle (Waples 1991). Gotceitas and Godin (1993) reported and reviewed evidence for strong responses by various salmonid species to sympatric predators (Metcalfe et al. 1987; Huntingford et al. 1988; Dill and Fraser 1984), but found only weak responses by juvenile salmon to an allopatric species, brook trout (*Salvelinus fontinalis*). Cook Creek hatchery fry in this study were descendants of steelhead that have been isolated as potential prey for sculpins, probably for several generations. In fact, by the nature of the hatchery program, hatchery steelhead would only encounter sculpin during seaward migration as smolts or returning adults and might be likely predators on sculpin, but certainly not prey for them. Thus, my results are consistent with the hypothesis that relaxation of benthic predation pressure during the juvenile stages, over several generations, may have increased the frequency of predator-susceptible phenotypes in the Cook Creek population relative to the wild Quinault population. Johnsson (1993) suggested that the combination of selection for rapid growth (which may have occurred in the Cook Creek population) and relaxed predation pressures in hatcheries will favor phenotypes that increase risk-taking behavior to maximize energy gain. Such a strategy would likely increase vulnerability of steelhead to predation in natural streams.

Qualitative observations of sculpin (lie-in-wait predators) and steelhead fry behavior provided some insight as to possible behavioral mechanisms that may have been responsible for the observed differences in predator avoidance ability. Most attacks by sculpin were preceded by the sculpin either making short abrupt movements when stalking a steelhead or quick flinches made during false, incomplete, or interrupted attacks. Steelhead appeared to be unaware of the threat posed by completely motionless sculpin and were only able to detect the presence of sculpin by this movement. Fry that did not overtly respond to these cues were eaten much more often than those that responded by darting away, suggesting that differences in the ability of fry to detect and (or) recognize short, quick, preattack movements of sculpin were important. In addition, data from the conditioning trials revealed that sculpin failed on a high percentage (63%) of attacks on naive sacrificial steelhead. The ability of steelhead fry to avoid sculpin, therefore, may also depend upon interrelated factors such as morphology and swimming speed (see Webb 1977, 1978; Taylor and McPhail 1985), although I have no evidence suggesting that differences in such factors existed between the two populations.

Experience can significantly improve the predator avoidance ability of juvenile salmonids (Thompson 1966; Ginetz and Larkin 1976; Patten 1977; Olla and Davis 1989), but the benefits gained by experience may be limited by genetically determined predator-avoidance capabilities. In this study, population type and experience were both factors affecting the ability of size-matched steelhead fry to avoid predation by sculpin. Wild fry with 50 min of visual exposure to predation suffered the lowest number of mortalities whereas hatchery-reared naive fry suffered the greatest mortality. Exposing hatchery-reared juveniles to predation probably provides the stimuli necessary to trigger innate avoidance responses. Consequently, conditioning juvenile salmonids to avoid predators may be a useful tool for hatchery managers attempting to improve postrelease survival in fry supplementation programs. With the exception of Thompson (1966), however, no published studies have investigated the effectiveness or the feasibility of training fish to avoid predators on a production scale. Such practices might improve postrelease survival and could be particularly beneficial for supplementation programs involving wild brood stock. However, the potential for training offspring of domesticated salmonid populations to avoid predators may be limited when innate responses have been altered through domestication and are inappropriate in natural environments.

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Total dissolved mercury in the water column of several natural and artificial aquatic systems of Northern Quebec (Canada)[1]

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Abstract: Total dissolved mercury concentrations in the water column of the La Grande-2 and Laforge-1 hydroelectric reservoirs and four neighbouring lakes near James Bay, northern Quebec, were measured to establish the impacts of extensive flooding of terrestrial environments on this potential vector of contamination to the aquatic biota. During three field visits between June and October 1993, while the sites were free from ice cover and the water column was not strongly stratified, filtered water samples were collected from multiple depths at various locations within both reservoirs and the four lakes. To compare the diverse subenvironments within the reservoir systems, sampling sites were selected to represent differences in (*i*) type of flooded soil, (*ii*) impoundment history, and (*iii*) water depth. At all stations total dissolved mercury concentrations were nearly constant, with an average value of 2.30 ng·L^{-1}, and a standard error of 0.04 ng·L^{-1}. Furthermore, reservoir concentrations were not statistically different from those of the lakes. Hence, we propose that the dissolved component in the water column does not play a significant role in the transfer of inorganic mercury to the aquatic food chain in recently developed hydroelectric reservoirs.

Résumé : L'importance de la colonne d'eau comme vecteur potentiel de mercure vers des organismes aquatiques suite à l'ennoiement d'environnements terrestres a été étudiée. Les concentrations de mercure total dissous dans deux réservoirs hydro-électriques, La Grande-2 et Laforge-1, ainsi que pour quatre lacs environnants situés près de la baie James, le moyen-nord québécois, ont été déterminées. Au cours de l'année 1993, trois campagnes d'échantillonnage furent effectuées entre les mois de juin et d'octobre, alors qu'une couverture de glace était absente et que la colonne d'eau était peu ou pas stratifiée. Des échantillons d'eau filtrée ont été receuillis à des profondeurs variables et à divers endroits pour chacun des lieux d'échantillonnage. Afin de mettre en évidence les différents sous-environnements au sein des réservoirs, les sites d'échantillonnage ont été choisis selon (*i*) le type de sol ennoyé, (*ii*) la période de mise en eau, et (*iii*) la profondeur. Les concentrations de mercure total dissous mesurées à chacun des sites sont semblables, avec une valeur moyenne de 2,30 ng·L^{-1} et une erreur standard de 0,04 ng·L^{-1}. De plus, les concentrations dans les réservoirs ne sont pas statistiquement différentes de celles mesurées dans les lacs. Nous suggérons que la composante dissoute de la colonne d'eau n'intervient pas de façon significative dans le mécanisme de transfert du mercure inorganique vers la chaîne alimentaire aquatique suite à la mise en eau des réservoirs hydro-électriques.

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Introduction

Numerous researchers working in remote, temperate regions have reported elevated concentrations of mercury (Hg) in fish of both lakes (Björklund et al. 1984; Swain and Helwig 1989; Håkanson et al. 1990; Wren et al. 1991) and hydroelectric reservoirs (Bodaly et al. 1984; Hecky et al. 1991; Jackson 1991; Verdon et al. 1991; James Bay Mercury Committee 1993). It is well documented that natural and anthropogenic Hg is widely dispersed via atmospheric transport (Slemr and Langer 1992; Lucotte et al. 1995) and subsequently accumulated in the humic horizon of soils following wet and dry deposition (Lee and Hultberg 1990; Aastrup et al. 1991; Dmytriw et al. 1995; Grondin et al. 1995). A large proportion of Hg found in natural

aquatic systems originates from catchment soils and is transported via runoff (Johannson et al. 1991; Mierle and Ingram 1991; Swain et al. 1992; Lucotte et al. 1995). In flooded systems, the same runoff dynamics operate, but internally the system is complicated by reactions stimulated by the degradation of organic matter at the newly established flooded soil–water interface. The remobilization of Hg from flooded forest soils to the various compartments of the aquatic system in response to these reactions has not yet been studied in detail, and, in particular, the importance of the dissolved fraction as a vector for Hg to the food chain remains to be confirmed. Although determinations of Hg in the aquatic biota were not included in the present investigation, its impetus derives from the above-mentioned uncertainties, in addition to an initial hypothesis that, given the strong affinity of Hg for particulate and dissolved organic matter, we would find elevated concentrations of total dissolved mercury ($[Hg_T]_D$) in reservoirs as compared with lakes.

Until recently, water column studies of Hg were hampered by difficulties associated with the sampling and analysis of subnanogram levels of total Hg (Hg_T). Analytical advances (Bloom and Crecelius 1983; Bloom and Fitzgerald 1988) have improved detection limits for the determination of Hg_T in water, while increased awareness of the necessity for ultraclean techniques during sample collection has considerably reduced problems associated with contamination. Although the results of several studies have been published for lakes in Wisconsin (Fitzgerald and Watras 1989; Wiener et al. 1990; Hurley et al. 1991, 1994) and elsewhere (Jackson 1986; Schintu et al. 1989; Lee and Iverfeldt 1991; Meili et al. 1991; St. Louis et al. 1994), reliable measurements of Hg_T in freshwater environments are not abundant. This is especially true for the extensive reservoirs developed for hydroelectric projects.

The objectives of this investigation were (*i*) to characterize, for the first time, the distribution of $[Hg_T]_D$ in the water column of hydroelectric reservoirs and natural lakes, (*ii*) to compare the findings with other recent studies (the results of which are based predominantly on unfiltered samples), and (*iii*) to assess, qualitatively, the importance of the dissolved fraction as a vector of Hg to aquatic biota of recently flooded systems. Although the cycling of methyl mercury (CH_3Hg^+) in aquatic environments is of importance with respect to bioaccumulation, here we report only total dissolved mercury ($Hg_{T\text{-}D}$) because the concentrations are very low in both reservoirs and lakes, and because preliminary results indicate that the proportion of $CH_3Hg^+{}_D$ (dissolved methyl mercury) making up $[Hg_T]_D$ does not exceed about 20% (S. Montgomery, A. Mucci, M. Lucotte, and P. Pichet, unpublished data).

Study area

The study area (Fig. 1) is located in the James Bay region of northern Quebec, on the granitic bedrock of the Precambrian Canadian Shield. Two hydroelectric complexes developed on the La Grande and Laforge rivers, LG-2 and LA-1, respectively, were chosen as representative of artificial systems. The inundation of 2630 km^2 of land to create the LG-2 Reservoir, located at 53°N, 77°W, occurred between 1978 and 1979. The LA-1 Reservoir, approximately 400 km east of LG-2, has a much shorter inundation history. Impoundment of LA-1 (1288 km^2) began in August 1993, with the maximum water level being attained by October 1993. Some areas, presently outside the immediate perimeter of the reservoir, were flooded during construction of the containment basin. One such zone is included in our study. It is located next to one of the reservoir dikes, LA-40, and was inundated in June 1992. Four sampling stations (4, 8, 121, and 122) are located in this area.

To compare results obtained for artificial systems to those of natural aquatic environments, water samples were collected from lakes in the vicinity of the reservoirs. Lakes Duncan and Detcheverry (stations 29 and 111, respectively) are situated near the LG-2 Reservoir whereas lakes Jobert and des Vœux (stations 124 and 123, respectively) are in the LA-1 area (Fig. 1). The water level of Jobert Lake was raised by about 6 m in the fall of 1993 after the lake was incorporated into the LA-1 Reservoir.

Materials and methods

Cleaning protocol

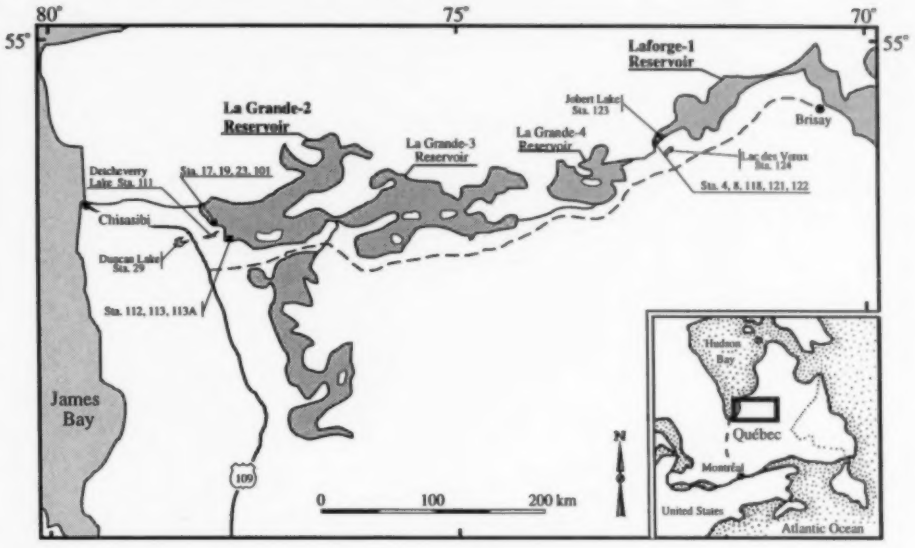
In preparation for the field excursions, a rigorous cleaning protocol was undertaken for all water sampling equipment. Great care was taken to avoid sample contamination. All containers used for sampling were made of Teflon® and were prewashed following a multistep procedure. First, they were soaked for several hours in a cold 7% NaOH (sodium hydroxide) solution to remove any organic matter adsorbed by the walls of the bottle, and then rinsed with NANOpure® deionized distilled water. The bottles were then filled with quartz-distilled 1 M HCl (hydrochloric acid), loosely capped, and heated overnight at 60°C. Following a rinse with NANOpure® water, the previous step was repeated. At the end of the second acid wash, the bottles were thoroughly rinsed, capped, and then individually placed in resealable plastic bags, each of which was sealed inside another bag. All other equipment used during collection (e.g., tubing, filter holders) was soaked overnight in a cold 1 M HCl solution, rinsed with NANOpure® water, and stored in resealable plastic bags.

Sampling

Sampling was conducted in an equally strict manner, with all possible precautions taken to prevent sample contamination. Water was collected using a manually operated peristaltic pump and a short length of Masterflex® silicone tubing to which was attached, at the outlet, a 47-mm Millipore in-line filter holder assembly. For each sample, two filters, a glass fibre (GF) filter and a 0.45-μm GN-6 mixed cellulose ester Gelman filter, were placed one atop the other in the filter unit. The GF filter was used as a prefilter to prevent rapid clogging of the GN-6 filter. On the basis of prior laboratory testing, we found washing the filters to be unnecessary. They were, however, rinsed at the time of sampling with approximately 250 mL of sample water.

Although the Teflon® bottles were never completely removed from their double bag enclosures, clean, nonpowdered PVC (polyvinyl chloride) gloves were worn at all

Fig. 1. Study area with locations of sampling stations.



times during sampling by the person uncapping, rinsing, filling, and resealing the sample containers. The bottles were kept in a cooler with ice packs until arrival at the field laboratory, where they were acidified (0.1% v/v) with high purity 6 M quartz-distilled HCl. All $Hg_{T\text{-}D}$ analyses were performed within 12 hours of sampling.

Three different samplers were used, depending on the sampling site. Four stations in the LG-2 Reservoir and two at LA-1 were sampled using an in situ multiport sampler designed for close-interval sampling in shallow areas (approximately 2 m deep). The main body of the sampler is a length of Plexiglas pipe (inside diameter, i.d. = 127 mm) into which lengths of Tygon® tubing (i.d. = 3 mm) were fixed at 25-cm intervals. Each sampler was installed vertically in the water, with its base resting at the flooded soil–water interface and the Tygon® sampling ports accessible from the water surface. Using the peristaltic pump, water was drawn through the Tygon® tubing from the desired depth, and following extensive rinsing, the samples were collected. The samplers remained in place for the duration of the sampling season.

In areas where water depths exceeded 13 m, sampling was conducted with the use of a 1-L Teflon® Kemmerer bottle. Subsampling of the water in the bottle was accomplished by attaching the inlet of the peristaltic pump to a valve at the bottle's base. In shallower environments, samples were collected by connecting the peristaltic pump to a Teflon® sampling hose, weighted at the inlet and lowered to the desired depth.

All sampling was conducted at multiple depths in the reservoirs, over various types of flooded soils. The three major soil types in the inundated zones are podzols, organic-rich soils, and peat bog soils, with podzols predominating (Grondin et al. 1995). A complete list of the sampling sites visited during the three 1993 field trips is presented in Table 1.

In addition to water sampling for $Hg_{T\text{-}D}$, samples were collected concomitantly for the determination of dissolved organic carbon (DOC). Furthermore, at the time of sample collection, several chemical and physical parameters were measured to characterize the water column. These included vertical profiles of temperature (T) and dissolved oxygen concentration ($[O_2]_D$) as well as measurements of pH and redox potential (Eh). Profiles of T and $[O_2]_D$ were obtained at 0.5-m intervals using a YSI model 57 water quality monitoring unit. Values for pH were determined with a gel-filled combination electrode with Ag/AgCl (silver – silver chloride) reference, while Eh was measured using a platinum – Ag/AgCl combination electrode, calibrated against a ferro–ferricyanate solution ($Eh_{Ag/AgCl}$ = +242 mV). The general limnological characteristics of the lakes and reservoirs sampled during the three 1993 field visits are shown in Table 2.

Analysis

All determinations of $[Hg_T]_D$ were conducted in the field laboratory by cold vapour atomic fluorescence spectrometry (CVAFS), following a modification of the technique perfected by Bloom and Fitzgerald (1988). In accordance with their method, dissolved Hg(II) is reduced to elemental Hg (Hg^0) vapour by a tin chloride ($SnCl_2$) solution in a reaction vessel, and Hg^0 analyzed by CVAFS. Our system, however, involves direct volatilization and therefore does not include a preconcentration step with a gold trap.

Briefly, samples are pretreated using a photochemical digestion prior to analysis to liberate and oxidize Hg(II) associated with stable organic complexes. Two 10-mL aliquots of each sample are transferred to precleaned quartz tubes. To each tube, 100 μL of the oxidizing agent, a 50 $g \cdot L^{-1}$ potassium persulfate solution, is added. The tubes are sealed with Parafilm® and stirred, and the solution is subjected to ultraviolet oxidation for 20 min, in a photochemical reactor. The

Table 1. Type of flooded soil or sediment underlying each sampling station, the inundation history for reservoir stations, and the corresponding maximum water column depth.

Station	Reservoir or lake	Type of flooded soil or sediment	Inundation history	Maximum water column depth (m)
17	LG-2	Podzol	14 years	2.5
19	LG-2	Peat bog	14 years	2
23	LG-2	Organic-rich soil	14 years	2.75
101	LG-2	Organic-rich soil	14 years	1.25
112	LG-2	Peat bog	14 years	7
113	LG-2	Podzol	14 years	12
113A	LG-2	Podzol	14 years	14
4	LA-1	Peat bog	1 year	0.75
8	LA-1	Podzol	1 year	1.75
121	LA-1	Stream	1 year	2
122	LA-1	Stream	1 year	1.25
118	LA-1	Peat bog	6 weeks	19.5
123	Jobert	Lacustrine		6.5[a]
	LA-1		6 weeks	13[b]
124	des Vœux	Lacustrine		5
29	Duncan	Lacustrine		6
111	Detcheverry	Lacustrine		7.5

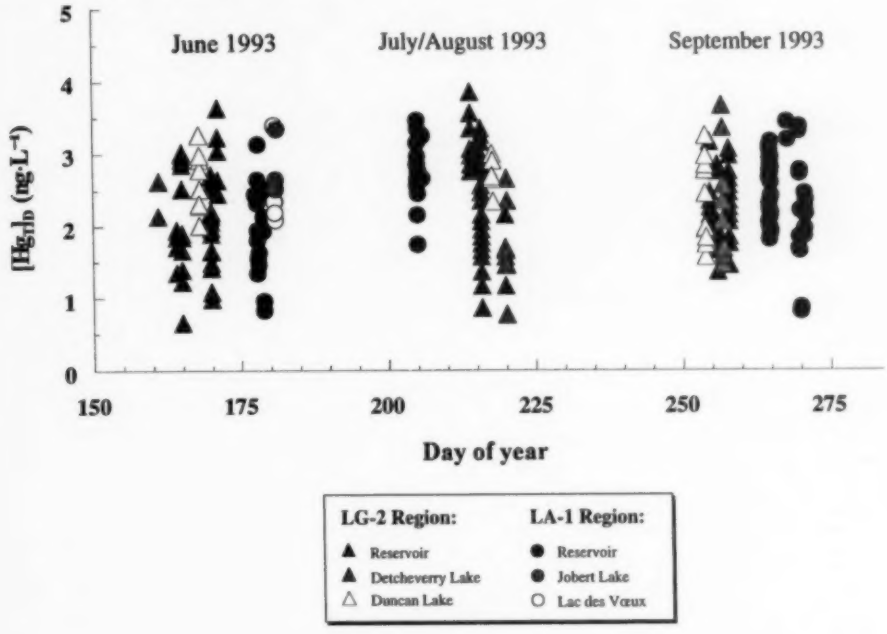
[a]Pre-inundation value.
[b]Post-inundation value.

Table 2. General limnological characteristics of the lakes and reservoirs sampled during the three 1993 field visits.

Lake or reservoir	pH	$Eh_{Ag/AgCl}$ (mV)	T (°C)	$[O_2]_D$ ($mg \cdot L^{-1}$)	[DOC] ($mg \cdot L^{-1}$)	$[SO_4]$ ($\mu mol \cdot L^{-1}$)
Duncan						
A	6.2	234	15	10.2	—	—
B	6.9	167	19	7.0	8.3	46.4
C	—	192	8	10.2	7.4	47.5
Detcheverry						
A	6.8	250	8–12	12.4	—	—
B	—	—	—	—	5.9	56.9
C	—	189	10	8.5	5.7	—
des Vœux						
A	6.9	—	14	11	1.9	—
B	—	264	—	—	2.3	23.2
Jobert						
A	6.8	—	15	10	3.4	—
B	5.5	243	18	8.5	2.8	30.2
LG-2						
A	6.6	293	11.5	9.2	8.0	—
B	6.0	240	16.9	6.0	7.3	34.7
C	6.4	172	8.7	9.4	7.5	51.0
LA-1						
A	6.0	302	14.5	7.7	4.8	
B	6.1	279	19.1	6.0	3.8	40.3
C	6.2	215	5.9	10.0	5.4	17.4

Note: A, June; B, July–August; C, September; —, data not available.

Fig. 2. Total dissolved mercury concentrations, ($[Hg_T]_D$, $ng \cdot L^{-1}$) in the water column. Data points for reservoir samples represent 14 years of impoundment in the case of La Grande-2 (LG-2) and 6 weeks to 1 year for Laforge-1 (LA-1).



reaction vessel, a 30-mL Teflon® centrifugation tube, is continuously bubbled with a Hg-free stream of argon (Ar) gas and is prepared by injecting 2 mL of the $SnCl_2$ solution, 0.5 mL of 6 M distilled HCl, and 0.5 mL of NANOpure® water. Following a 4-mL injection, Hg^0 is stripped out of the reactor and carried in an Ar gas stream, at an optimal flow of 200 $mL \cdot min^{-1}$, to the fluorescence cell, where it is excited by a 4-W germicidal Hg vapour fluorescent tube. The upper part of the reduction chamber and the fluorescence cell are maintained at 50–70°C to avoid water condensation. The amplifier output is converted (1 conversion/s) by an analog digital converter having at least a 15-bit resolution. The converted data are collected and analyzed on an Amiga computer (1000 or 3000 model, Commodore Corporation) using software developed in our laboratory. The concentration of Hg is calculated from the area of the fluorescence peak. The detection limit for the method is 0.3 $ng \cdot L^{-1}$, with procedural blanks of 0.5 $ng \cdot L^{-1}$.

DOC was measured as CO_2 using a modified photochemical oxidation unit consisting of a quartz reaction chamber and a 40-W, low pressure, six-coil UV lamp (Dohrmann model DC-80) in the presence of persulfate (Bauer et al. 1991).

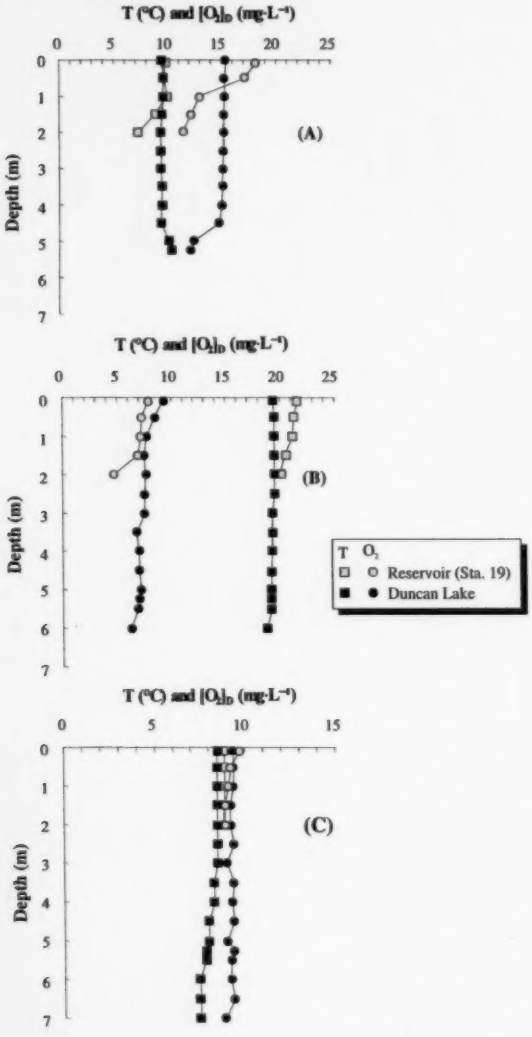
Results

For all sampling stations, $[Hg_T]_D$ values were nearly constant with an average value of 2.30 $ng \cdot L^{-1}$ (n = 238, SE = 0.04 $ng \cdot L^{-1}$, max/min = 3.87/0.64 $ng \cdot L^{-1}$). A plot of these results, with respect to time (day of year), is presented in Fig. 2, while Table 3 shows the general findings for the three field visits and the different sampling areas (LG-2, LA-1, and lakes). The results of t tests (α = 0.05) indicate that no statistical difference in $[Hg_T]_D$ can be noted between the LG-2 and LA-1 reservoirs and the four lakes. In addition, no significant seasonal variation (α = 0.05) was observed for the following cases: (*i*) LG-2, June vs. September and July–August vs. September; (*ii*) LA-1, June vs. September; and (*iii*) lakes Duncan and Detcheverry, all three sampling campaigns. For the remaining three reservoir cases (i.e., June vs. July–August for LG-2 and LA-1, and July–August vs. September for LA-1) the concentration variability can only be discounted at a significance level of α = 0.01. Insufficient data are available for lakes Jobert and des Vœux.

Vertical profiles of T and $[O_2]_D$ indicate that, in nearly all cases, the water column was well mixed (Fig. 3). Weak thermal stratification was observed during the June visit at several sampling sites in the LG-2 Reservoir and Detcheverry Lake in the same region. The water column was always oxygenated (Table 2).

Dissolved organic carbon concentrations, [DOC], measured in lake samples range from 1.7 to 8.3 $mg \cdot L^{-1}$, with an average of 5.1 $mg \cdot L^{-1}$ (n = 26). Values for the reservoir samples have a mean of 7.9 $mg \cdot L^{-1}$ and show much greater variability with a minimum value of 2.4 $mg \cdot L^{-1}$ and an exceptional maximum concentration of 29.4 $mg \cdot L^{-1}$. Considering the two reservoirs separately, we find that [DOC] values for the younger LA-1 Reservoir are comparable to those of lakes Detcheverry, Jobert, and des Vœux, with an average of 4.8 $mg \cdot L^{-1}$ and a variability of

Fig. 3. Profiles of water column temperature, *T*, (°C) and dissolved oxygen concentration, $[O_2]_D$, (mg·L^{-1}) representative of the general conditions encountered during the three 1993 sampling visits: (A) June, (B) July–August, and (C) September.



7.9 mg·L^{-1} (Table 2). In contrast, [DOC] values for LG-2 are approximately twice as high (average = 8.9 mg·L^{-1} and max/min = 29.4/4.8 mg·L^{-1}). Figure 4 presents six profiles of $[Hg_T]_D$ and [DOC] representative of the stations sampled.

Discussion

The $[Hg_T]_D$ values measured in this study, for both lake and reservoir samples, are comparable to available published data for natural freshwater systems (Fitzgerald and Watras 1989; Gill and Bruland 1990; Lee and Hultberg 1990; Mierle 1990; Wiener et al. 1990; Hurley et al. 1991; Driscoll et al. 1994; St. Louis et al. 1994). Disregarding $[Hg_T]$ cited in investigations that predate the strict, ultraclean sampling protocols developed by Gill and Fitzgerald (1985) and the analytical improvements of Bloom and Fitzgerald (1988), literature values for $[Hg_T]$ in waters not influenced by point-source contamination generally range from 1 to 13 ng·L^{-1}. For two distinct freshwater ecosystems, we observed a smaller variation, 3.23 ng·L^{-1}, for the combined lake and reservoir results.

Table 3. General results of $Hg_{T\text{-}D}$ analyses for the 1993 water sampling.

	A	B	C
LG-2 reservoir			
Average	2.07	2.41	2.28
SE	0.10	0.11	0.07
Max/min	3.65/0.64	3.87/0.87	3.17/1.37
Number of analyses	43	45	38
LA-1 reservoir			
Average	2.02	2.60	2.31
SE	0.15	0.10	0.10
Max/min	3.15/1.35	2.97/1.77	3.38/0.83
Number of analyses	13	12	34
Natural lakes			
Average	2.60	2.19	2.38
SE	0.10	0.21	0.13
Max/min	3.35/2.01	2.97/0.77	3.67/1.47
Number of analyses	18	11	24

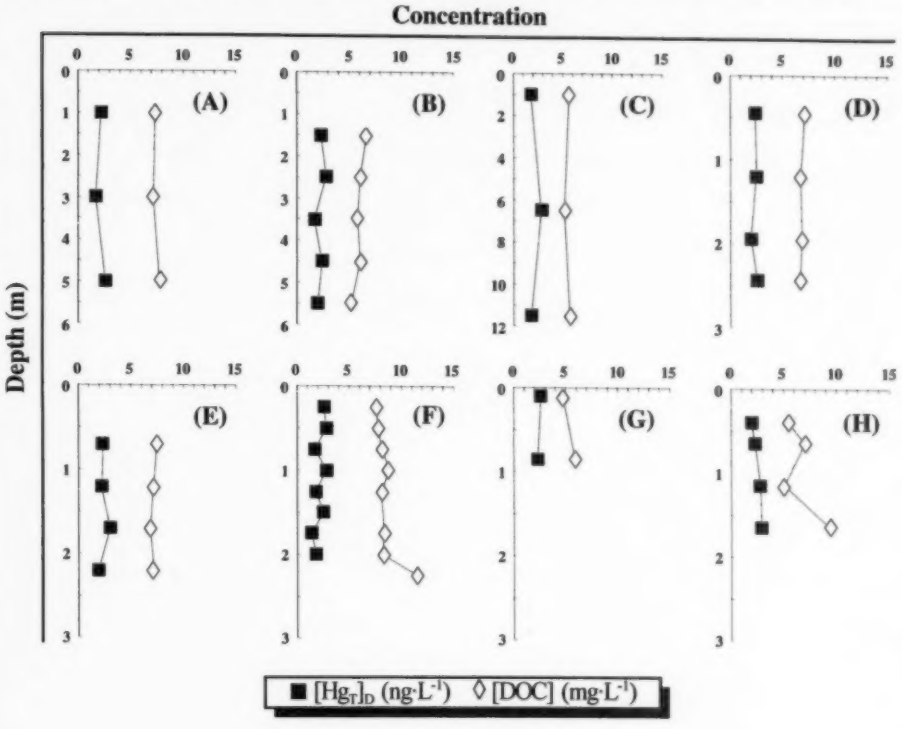
Note: The three field trips, June, July–August, and September, are represented by A, B, and C, respectively. The units for the average, SE, and max/min are ng·L^{-1}.

Natural lakes

The $[Hg_T]_D$ measured in filtered (0.45-μm) samples collected at multiple depths from the four lakes range from 0.77 to 2.90 ng·L^{-1}. The average concentration for the 31 samples is 2.39 ng·L^{-1}. In a similar context, several researchers (Fitzgerald and Watras 1989; Wiener et al. 1990; Hurley et al. 1991) participated in an extensive study of natural lakes in northcentral Wisconsin. Fitzgerald and Watras (1989) collected unfiltered surface waters from eight lakes during the autumn mixis. In four of the lakes, $[Hg_T]$ in the water ranged from 0.9 to 2 ng·L^{-1}. The work of Hurley et al. (1991) was carried out in the artificially acidified (pH = 5.6) basin of Little Rock Lake, where they collected unfiltered and filtered (0.7-μm) samples from the epilimnion and anoxic hypolimnion. For samples recovered from the oxygenated water column a range similar to that reported by Fitzgerald and Watras (1989) and in this study was found.

Measurements of $[Hg_T]$ in water have also been reported for Swedish freshwater lakes (Lee and Hultberg 1990; Johansson et al. 1991; Lee and Iverfeldt 1991; Meili 1991) and for waters flowing from four different catchments at the Experimental Lakes Area (ELA), in northwestern Ontario (St. Louis et al. 1994). The concentration range presented for unfiltered samples from lakes in Sweden is fairly wide, from about 2 to 12 ng·L^{-1}, as compared with

Fig. 4. Concentration profiles of dissolved organic carbon, [DOC], ($mg \cdot L^{-1}$) and total dissolved mercury, $[Hg_T]_D$, ($ng \cdot L^{-1}$) in the water column of (A) Detcheverry Lake, (B) Station 112, (C) Station 113, (D) Station 17, (E) Station 19, (F) Station 23, (G) Station 4, and (H) Station 8.



an overall variation of about 3 $ng \cdot L^{-1}$ for our study. In a comprehensive review of recent research on Hg in the Swedish environment, the variability of water data is attributed to seasonal changes, with $[Hg_T]$ usually being higher in spring and autumn than in summer (Lindqvist 1991). The results of the ELA investigation are considerably more variable. Values of $[Hg_T]$ for samples collected at outflow points of four catchments (when flow was sufficient for sampling) range from relatively high values, 10 to 16.3 $ng \cdot L^{-1}$, from an upland terrain, to lower values, 0.15 to 3.5 $ng \cdot L^{-1}$, for the outflow of a lake catchment. Following a similar trend to that observed for Swedish lakes, the data set for the ELA project, in the majority of instances, shows elevated levels during spring and autumn.

Reservoirs

With approximately 890 km^2 of flooded forest included in the 1288 km^2 (maximum) LA-1 Reservoir and 2630 km^2 of inundated terrain making up 95% of the surface of the LG-2 Reservoir, there is resuspension of organic matter from the exposed shores during periods of high erosion (e.g., immediately following inundation or in response to water level fluctuations). As it is commonly accepted that the predominant input of Hg to terrestrial systems is atmospheric deposition and that Hg has a strong affinity for organic matter, one would expect to find elevated $[Hg_T]_D$ in the water of reservoirs, as compared with natural lakes. The findings of this study, however, demonstrate that regardless of the system sampled, $[Hg_T]_D$ values do not differ significantly. For the three sampling visits combined, the average concentrations calculated for LG-2, LA-1, and the lakes are 2.25, 2.31, and 2.39 $ng \cdot L^{-1}$, respectively. Of particular interest is the diversity of conditions under which sampling was conducted. During the sampling season (June to September) water column temperature fluctuations of over 10°C were observed (Fig. 3). Furthermore, the reservoir stations were selected specifically for the purpose of evaluating several factors that we deemed to be potentially influential for Hg levels: (*i*) type of flooded soil (podzol, organic-rich, or peat bog soil), (*ii*) impoundment history (6 weeks, 1 year, or 14 years), and (*iii*) water depth (0.85–19 m).

For both reservoirs, a slight increase in $[Hg_T]_D$ was noted for the July–August sampling visit. Also observed at 3 of the 12 sample stations were elevated levels of periphyton. As these algal communities represent a considerable Hg source (Bonzongo et al. 1993), and colonized our permanently installed sampling devices, it is probable that they could have biased our results through incorporation in the samples, either as a result of minute tears in the 0.45-μm filter caused by increased pumping pressure during sampling, or in response to cell lysis during filtration.

With the exception of this study, very few researchers have investigated the implications of extensive flooding on Hg levels in the water column. In a study carried out in the summer of 1993 the ELA research group initiated an experimental project to evaluate the effects of impoundment by raising the water level over a wetland terrain by 1.2 m to create a small reservoir. Preliminary analyses of unfiltered samples showed elevated [Hg_T] immediately following inundation (V.L. St. Louis, personal communication). The discrepancies between the results of the two studies may be related to the presence of Hg-rich particulates in the unfiltered samples.

Filtered versus unfiltered samples

Water column studies of sufficient duration to document seasonal changes (e.g., Lindqvist 1991; St. Louis et al. 1994) have reported [Hg_T] ranges in natural systems that are generally 4–5 times greater than the 2.9 ng·L^{-1} variability we observed. These variations are attributed to changes in the hydrological regime (increased or decreased runoff). As no temporal or spatial variations are apparent in our data set, the difference would seem to stem from a failure to distinguish suspended particulate matter from the dissolved fraction.

The relationship between concentrations of suspended matter and Hg_T in the water has been documented by numerous researchers (Cranston and Buckley 1972; Frenet 1981; Nishimura and Kumagai 1983; Ferrara et al. 1986; 1989). Gill and Bruland (1990) reasoned that elevated Hg levels determined during a February lake water sampling were the result of enhanced resuspension of bottom sediments from winter storms. Ferrara et al. (1991) concluded from the study of a naturally contaminated system that the aqueous transport of dissolved Hg was insignificant as compared with that effected by suspended particulates. This is further substantiated by recent work of Mucci et al. (1995) who show that, in the water column, Hg associated with suspended particulate matter represents a significant fraction of the total Hg pool. Finally, from an investigation conducted in the Onondaga Lake area of New York, Bloom and Effler (1990) present results for [Hg_T] in the water for both filtered (0.2-μm) and unfiltered samples. Measured concentrations for filtered samples were consistently found to be 20 to 50% lower than those for unfiltered water.

The effect of suspended particulate matter in a reservoir setting is expected to be even more pronounced than for lakes as a result of the potential for tremendous influxes of solid organic material. Though no difference in the $[Hg_T]_D$ was observed in waters collected from the two northern Quebec reservoirs, increases in [Hg_T] were reported following inundation of a small wetland (V.L. St. Louis, personal communication). We suggest that an elevated particulate load may account for the variation. This is substantiated by Mucci et al. (1995) who report that during a resuspension experiment, the proportion of the total Hg burden of the water column that is made up of particulate Hg increased from 20 to 80%.

Influence of dissolved organic carbon

Numerous researchers have documented that there exists a positive correlation between inorganic Hg and DOC concentrations in natural waters (Andren and Harriss 1975; Lodenius et al. 1987; Jackson 1989; Xu and Allard 1991; Miskimmin et al. 1992). From our Hg data set, comprised solely of the dissolved fraction, we see no consistent relationship between [DOC] and $[Hg_T]_D$ (Fig. 4). Although $[Hg_T]_D$ for LG-2 and LA-1 are not statistically different, [DOC] reached elevated levels of up to 29.4 mg·L^{-1} in the older LG-2 Reservoir, while in the LA-1 Reservoir, [DOC] are about two times less. Differences in residence times or inundation histories may explain the DOC variability observed in the two reservoirs.

Because organic matter has a great affinity for Hg, the previously reported correlation between [DOC] and [Hg_T] in aquatic systems is likely a result of the failure to make the distinction between the Hg associated with the dissolved and particulate organic fractions. For example, during a recent investigation of Adirondack lakes having [DOC] ranging from 3.4 to 26.5 mg C·L^{-1}, Driscoll et al. (1994) observed no relationship between $[Hg_T]_D$ and [DOC], while the particulate fraction showed a strong correlation with [DOC].

Conclusion

The main conclusions to be drawn from the available data set are that there is no significant variation in $[Hg_T]_D$ regardless of (*i*) aquatic system (artificial vs. natural), (*ii*) type of flooded soil (podzol, organic-rich, or peat bog soil), (*iii*) duration of impoundment (6 weeks, 1 year, or 14 years), (*iv*) depth of water (0.85 to 19 m), or (*v*) seasonal changes. For the period in question, during which the water column was always oxygenated and free of ice cover, $[Hg_T]_D$ values were always low, remaining at about 2.30 ng·L^{-1} with a variability of 0.64 to 3.87 ng·L^{-1}. Observed increases of [Hg_T] measured on unfiltered water samples, during periods of high overland flow or following flooding, can probably be attributed to elevated levels of suspended matter and therefore reflect a failure to distinguish particulate and dissolved fractions. Although the water is an important vector for Hg-laden particulates (Ferrara et al. 1989; Mucci et al. 1995), the dissolved component does not appear to play a significant role in the contamination of the aquatic food chain observed in recently developed hydroelectric reservoirs. That is, on the basis of the preliminary results for $[CH_3Hg^+]_D$ of this investigation (unpublished data) and the observations of other researchers (Schintu et al. 1989; Lee and Iverfeldt 1991; Driscoll et al. 1994; St. Louis et al. 1994), the proportion of CH_3Hg^+ making up [Hg_T] is generally limited to between 1 and 20%. In view of this percentage and the small range of $[Hg_T]_D$ observed in the two reservoirs and four lakes, the potential contribution of CH_3Hg^+ from this fraction to the biota is likely negligible. This conclusion is substantiated by the recent findings of Plourde et al. (1994) who, following a study in the same region, reported no significant difference between [CH_3Hg^+] in phytoplankton of the reservoirs and lakes, while [CH_3Hg^+] in zooplankton were about five times higher in the reservoirs. Furthermore, several researchers also working in the same region (Lebeau et al. 1994; Tremblay and Lucotte 1994) documented [CH_3Hg^+] determined for insects and periphyton to be significantly higher in the reservoirs than the lakes.

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Mercury and lead profiles and burdens in soils of Quebec (Canada) before and after flooding[1]

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Abstract: Forest soils and their flooded equivalents were sampled in the Cabonga (southern Quebec) and La Grande (northern Quebec) hydroelectric complexes and analyzed at 1-cm intervals for organic C, N, Fe, Mn, Hg, and Pb. In the La Grande region, thin *Cladina* sp. podzols and thicker *Pleurozium* sp. podzols are characterized by lower Hg burdens (2500 and 5100 $\mu g \cdot m^{-2}$, respectively) than the thicker but less dense soils typical of wetter environments (6000 to 8000 $\mu g \cdot m^{-2}$). These differences suggest that a fraction of Hg is leached downslope from uplands (podzols) and partially accumulates in poorly drained soils (wetlands) below. Unlike Pb, Hg showed no apparent decrease in atmospheric transport over 1000 km from south to north, away from the industrialized regions. Upon inundation, the oxidation–reduction potential drops rapidly but there was no noticeable decrease in the organic C burden, with the exception of flooded podzols where the surficial organic layer may undergo erosion. In flooded wetland soils, Hg, Pb, and Fe display a partial redistribution towards the sediment–water interface, but the Hg burdens of these soils remain comparable to those of their unflooded equivalents. In contrast, the surficial erosion of the flooded podzols may represent one pathway for Hg to enter organisms in the reservoir.

Résumé : Des sols forestier et leurs équivalents inondés furent échantillonnés dans les régions du réservoir Cabonga (sud du Québec) et du complexe La Grande (nord du Québec) et analysés selon une maille centimétrique pour déterminer leur teneurs et charges en C, N, Fe, Mn, Hg et Pb. Les podzols à cladine (mince) et les podzols à hypne (épais) échantillonnés dans la région du complexe La Grande sont les plus faiblement chargés en Hg (2500 et 5100 $\mu g \cdot m^{-2}$, respectivement) comparativement aux sols mésiques voisins (6000 à 8000 $\mu g \cdot m^{-2}$). Ces différences suggèrent qu'une fraction importante du Hg est lessivée des sols xériques en haut de pente vers les sols mésiques situés dans les dépressions. Contrairement au Pb, aucune diminution du Hg transporté via l'atmosphère est notée sur une distance de plus de 1000 km, des centres urbains méridionaux vers les hautes latitudes. Suite à l'inondation des sols, le potentiel d'oxydoréduction baisse rapidement mais la charge en C est similaire, sauf dans le cas des podzols, où la couche organique de surface peut être érodée. Dans les sols mésiques inondés, le Hg, le Pb et le Fe se redistribuent vers l'interface sédiment–eau. Malgré cette remobilisation, la charge en Hg y demeure toutefois comparable à celle de leurs équivalents exondés. Au contraire, l'érosion de la surface des podzols inondés peut représenter une des voies d'entrée du Hg chez les organismes des réservoirs.

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Introduction

Following inundation of soils during the development of hydroelectric reservoirs, mercury (Hg) is transferred from the flooded soils to aquatic biota. Hg contamination of organisms living in such reservoirs eventually reaches toxic levels, often exceeding 5 to 6 times the concentrations of Hg in natural freshwater ecosystems (Verdon et al. 1991). A direct relationship between the surface area of a reservoir and the levels of Hg accumulation in some fish species has been observed (Jackson 1991; Johnston et al. 1991).

Most studies of the geochemical behaviour of Hg have focused on unperturbed environments, e.g., forest soil or lake sediment ecosystems (Dudas and Pawluk 1976; Semu

et al. 1987; Arakel and Hongjun 1992; Young and Harvey 1992). It is thus difficult to extrapolate conclusions from these studies to the geochemical behaviour of Hg in transitional environments such as flooded soils. Recent studies, however, suggest that biodegradation of submerged organic material and the dissolution of labile organometallic compounds promote the methylation of mercury and its release into the water column (Jackson 1988; Morisson and Thérien 1991).

A few studies focused on determining the origin of Hg found in ecosystems located far from centres of industrial activity. In the La Grande area in northern Quebec, the high concentrations of this metal found in terrestrial and aquatic systems cannot directly be attributed to either nearby industrial pollution or major geological sources of Hg. Hg enrichment measured in numerous boreal lacustrine sediments has led scientists to believe that atmospheric emissions from industrial activities (e.g., combustion of coal, domestic wastes, and pharmaceuticals) can be transported thousands of kilometres away from their point of origin (Delisle et al. 1979; Ouellet and Jones 1982; Evans 1986; Rybak et al. 1989; Verta et al. 1989). The Hg is then absorbed by forest vegetation and ultimately incorporated into soil litter through leaf fall (Iverfeldt 1991; Lucotte et al. 1995). Quebec forest soils have been accumulating atmospheric deposits of anthropogenic Hg for approximately 50 years (Lucotte et al. 1995).

The affinity of Hg for soil organic matter has been observed by several authors (Dudas and Pawluk 1976; Gotoh and Koga 1977; Lodenius et al. 1987; Semu et al. 1987; Severson et al. 1992; Arakel and Hongjun 1992). In Scandinavia and Italy, the average concentration of Hg in the organic horizon of forest soils is reported to be 100–600 $ng \cdot g^{-1}$ (Lindqvist 1991; Ferrara et al. 1991; Meili et al. 1991; Mukherjee 1991). The adsorption of Hg by oxyhydroxides of Fe and Mn and by clay minerals appears to be of secondary importance, particularly in organic-rich soils (Dmytriw et al. 1995).

Upon inundation, forest soils are subjected to physical modifications, such as saturation of interstitial spaces and compaction under the weight of the overlying water column (Godshalk and Barko 1985; Gunnison et al. 1985). In addition, soils undergo numerous biochemical changes that may affect the metal binding capacity of soil components. These changes include an increase in pH resulting from the dilution of humic and fulvic acids and a fall in redox potential (Eh) through biodegradation of organic material accompanied by a reduction in Fe and Mn oxyhydroxides (Godshalk and Barko 1985; Gunnison et al. 1985).

The first goal of this study was to characterize Quebec boreal forest soils to provide the basis for elucidating Hg retention processes. By comparing concentrations and burdens of heavy metals in organic horizons of different soil types, the distribution of Hg is addressed at three different scales as follows: (*i*) microsystemic, considering Hg concentration profiles in particular soils; (*ii*) ecosystemic, considering Hg burdens in different soil types; and (*iii*) landscape, considering Hg profiles and burdens in soils more than 1000 km apart. The third category addresses the relative importance of anthropogenic Hg in proportion to naturally occurring Hg in north central Quebec.

The second goal of this paper was to elucidate the mechanisms responsible for the internal remobilization of soil Hg upon inundation and to evaluate its potential for release into the overlying water column. To accomplish this, we compared Hg concentrations and burdens in flooded soils with those in their pristine counterparts.

The originality of this research stems from the use of centimetric core sampling for Hg and subsequent integrated burden calculations for various types of soils representative of the northern Quebec pedology. We emphasize that difficulties in sampling flooded soils in hydroelectric reservoirs remain an inherent problem in this type of study: obscurity, cold, core penetration obstructed by root systems, standing dead trees, and potential contamination of the samples are major obstacles to overcome.

Study area

The field study was conducted during the summers of 1990, 1991, and 1992 in the Cabonga (47°N, 76°W) and La Grande regions (53°N, 77°W) of Quebec (Fig. 1). The Cabonga reservoir is located approximately 320 km northwest of Montréal on the Canadian Shield. The bedrock is composed of Precambrian-age igneous and metamorphic rocks. *Betula papyrifera* (white birch), *Picea mariana* (black spruce), *Acer saccharum* (sugar maple), and *Abies balsamea* (balsam) are the principal species of trees in the mixed forest of this region. The La Grande area, situated 1200 km north of Montréal, is underlain by Precambrian granite. The boreal forest is composed principally of *Picea mariana* and *Pinus banksiana* (grey pine) with *Cladina* sp. (caribou moss), *Sphagnum* sp., and *Pleurozium* sp. (sphagnum) comprising the shrub component of the forest.

Undulating relief and subarctic conditions are key factors in the evolution and composition of soils (Commission canadienne de pédologie 1976). Podzols, poorly drained wetland soils, and peat soils were the soil types sampled for this study. Podzols predominate in the Cabonga region (Énergie, Mines et Ressources Canada 1974), and over about 90% of the La Grande watershed. Podzol soils have an organic horizon (average C >40%) less than 15 cm thick. The transition to the mineral horizon (average C <17%) is abrupt. Accumulation of Fe oxyhydroxides in the illuviated B horizon is typical of these soils. While podzols are found in upland areas where the terrain is relatively well drained, wetland and peat soils predominate in poorly drained, low-lying areas. These soils cover less than 10% of the La Grande watershed. Their organic horizon can be more than 30 cm thick. Because we have grouped our different soil profiles according to several geochemical variables, our classification distinguishes between wetland and peat soils, although this difference is not recognized by the Canadian Pedologic Commission.

Materials and methods

Soil sampling

Samples of pristine forest soils were collected at random to lessen chances of sampling bias. The study area includes the entire La Grande complex from La Grande-1 (LG-1) to Laforge-1 (LA-1), as well as the watershed of the Cabonga

reservoir (Fig. 1). Pristine soil cores were collected manually along vertical sections with 15 cm diameter polyvinyl chloride (PVC) tubes.

Forest soils submerged for several years at shallow depths in hydroelectric reservoirs often lose their organic horizon through erosion by ice and waves (Louchouarn et al. 1993), but beaver ponds and small protected bays are excellent natural laboratories for studying trace metal remobilization following the submergence of soils. Such flooded soils are well preserved because water level fluctuations are minimal and shore erosion is limited. Soils flooded recently (within 1–3 years) were sampled in beaver ponds, while a small protected bay (Dike 24) in the LG-2 reservoir provided samples of soils flooded for 11–13 years. These perturbed soils were sampled manually along a vertical section by divers using 15 cm diameter PVC tubes.

In the field, pristine soils were subsampled along vertical sections at 1-cm intervals, heat-sealed in plastic bags, and frozen. For the flooded soils, pH and Eh were measured under a nitrogen atmosphere to preserve the prevailing redox conditions before the samples were heat-sealed in plastic bags, and frozen.

Laboratory analyses

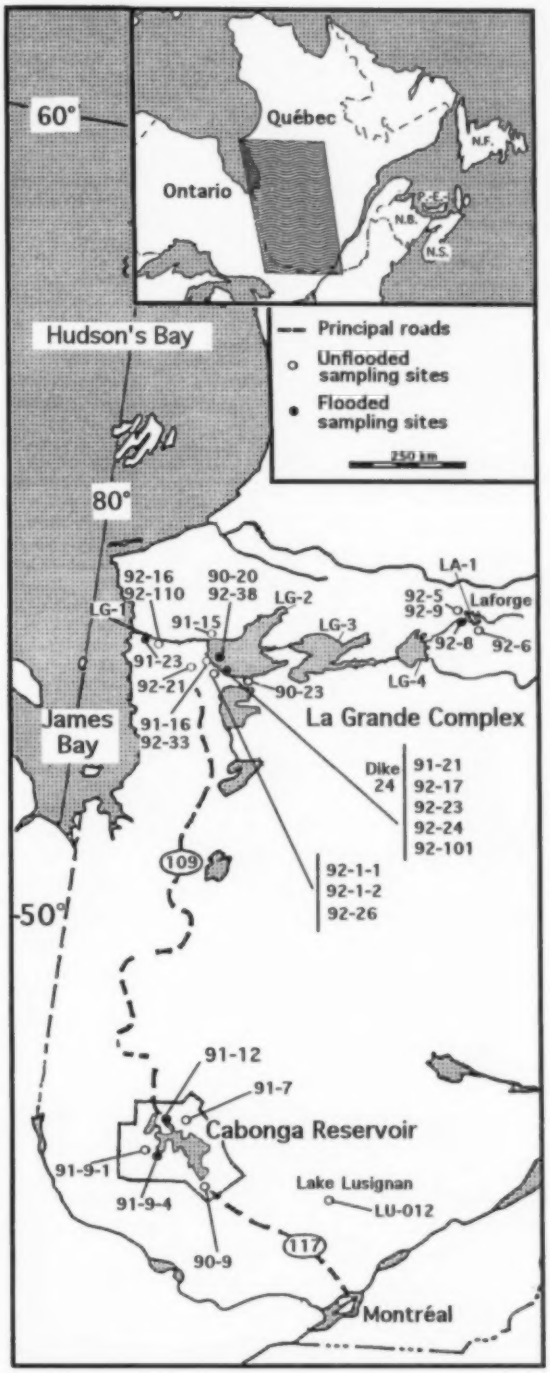
In the laboratory, 1 g of wet sediment was digested in a 10:1 solution of nitric (HNO_3) and hydrochloric (HCl) acids at 120°C for 6 h. Reagent contamination and the efficiency of the digestion procedure were gauged by digestion of National Research Council standards (BCSS-1 and MESS-1) and the use of reagent blanks. Total Hg concentrations were measured by atomic fluorescence (Louchouarn et al. 1993; Chaire de recherche en environnement Hydro-Québec/CRSNG/UQAM 1992, 1993). Concentrations as low as 0.2 $ng \cdot g^{-1}$ can be measured on subsamples of less than 1 g, with a reproducibility within 7%. Pb was analyzed by graphite furnace atomic absorption. Each 1-cm subsample was freeze-dried to measure the percentage of water, thereby allowing the expression of heavy metal concentrations on the basis of dry weight. The levels of organic C and N were measured using a CN Carlo Erba elemental analyzer with a reproducibility on the order of 1%. The burdens (B) of organic C, Hg, and Pb were evaluated throughout the organic horizons of the different soils. They were calculated according to the following equation:

$$B = \int_{x=1}^{n} ([E] \times \text{dry density})\, dx$$

where $[E]$ is the concentration of the element under consideration and x is the thickness, in centimetres, of the organic horizon. Operationally defined reactive Fe and Mn, extracted using a citrate–dithionite–bicarbonate method (Lucotte and d'Anglejan 1985), were analyzed by atomic absorption. The precision of the method is ±5%.

Three statistical treatments were used to evaluate the results. Total metal burdens and concentrations in pristine soils were compared with their flooded equivalents using a t test. Latitudinal differences in metal concentrations and burdens between Cabonga podzols and La Grande podzols were also examined by a t test. The ANOVA test

Fig. 1. Study region in northern Quebec and location of sampling sites.



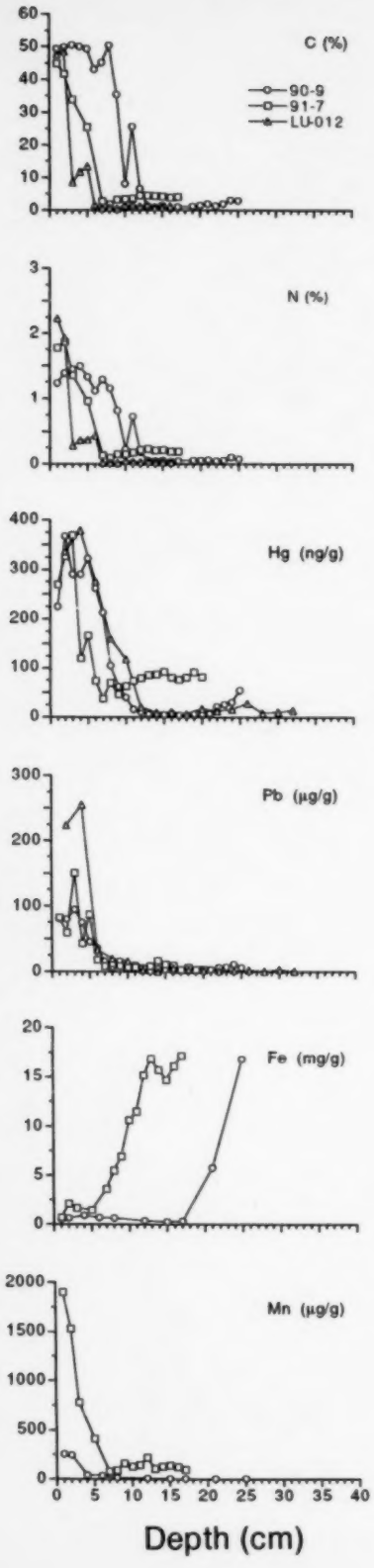


Fig. 2. C, N, Hg, Pb, Fe, and Mn profiles for pristine podzols sampled in the Cabonga watershed.

allowed us to verify if the differences in total Hg burden between soil types were attributable solely to sample heterogeneity. Finally, simple linear regressions served to describe and predict the relationship between heavy metals and organic C, and that of heavy metals and reactive Fe.

Results

Pristine soils

Podzols and wetland soils of Cabonga region are represented by cores 90-9, 91-7, and LU-012 (Fig. 2), and 91-9-1 (Fig. 3), respectively. The thickness of the organic horizon ranges between 4 and 25 cm. In the La Grande region, *Cladina* sp. podzols display a thin, living surficial cover (2 – 3 cm thickness) overlying a thin, undifferentiated organic horizon (cores 90-23, 91-1-1, 92-9, 92-110; Fig. 4*a*). *Pleurozium* sp. podzols (cores 91-15, 92-26, 92-33, Fig. 4*b*) and wetland soils (cores 92-6 and 92-16; Fig. 5*a*) have a living surface cover 2–4 cm thick. The underlying organic horizon is often partially degraded but is always composed of identifiable remains of *Pleurozium* sp. and (or) *Sphagnum* sp. moss. The organic horizon of the wetland soils, cores 92-1-2 and 92-21 (Fig. 5*b*), and the peat soil 91-16 (Fig. 5*c*) is at least 30 cm thick and comprised of a tightly interlaced network of identifiable dead *Sphagnum* sp. remains. In podzols, maximum concentrations of organic C and N occur at the surface of the core and decrease abruptly with depth. In contrast, pristine wetland and peat soils show N enrichment in the illuviated organic horizon located at the base of the cores.

The vertical distribution of Fe oxyhydroxides reveals clear differences among podzols, wetland soils, and peat soils. Fe oxyhydroxides accumulate in the mineral (B) horizon of podzols, the illuviated portion of the organic horizon of wetland soils, and near the top of the organic horizon of peat soils (Figs. 2–5). The acid conditions (pH = 4.5–5.2) and local reducing conditions in highly organic soils favour the presence of Mn in its reduced form (Mn^{2+}). These ions are very mobile and can be leached by percolating waters or reprecipitated near the surface (Jarvis 1984), as shown by the maximum concentration of Mn oxides in the first several centimetres of the pristine profiles studied.

In the 10 podzols studied (Figs. 2, 4*a*, and 4*b*), maximum concentrations of Hg and Pb are associated with the organic horizon (simple linear regression; Hg: $r^2 = 0.70$, $p < 0.001$; Pb: $r^2 = 0.62$, $p < 0.001$). The heavy metal profiles are independent of the reactive Fe profile (simple linear regression: Hg: $r^2 = 0.24$, $p > 0.01$; Pb: $r^2 = 0.43$, $p < 0.002$). The profiles of Hg and Pb in northern wetland soils are distinctly different from neighbouring podzols as well as the wetland soils in the Cabonga region (Figs. 3–5). Maximum Hg and Pb concentrations are recorded deeper within the core, i.e., at depths of 10–15 cm for Pb and 15–40 cm for Hg. These peak concentrations of heavy metals are found near the zones of highest N and Fe concentrations. In the pristine peat soil, Hg and Pb profiles are relatively uniform throughout the depth of the core (Fig. 5*c*).

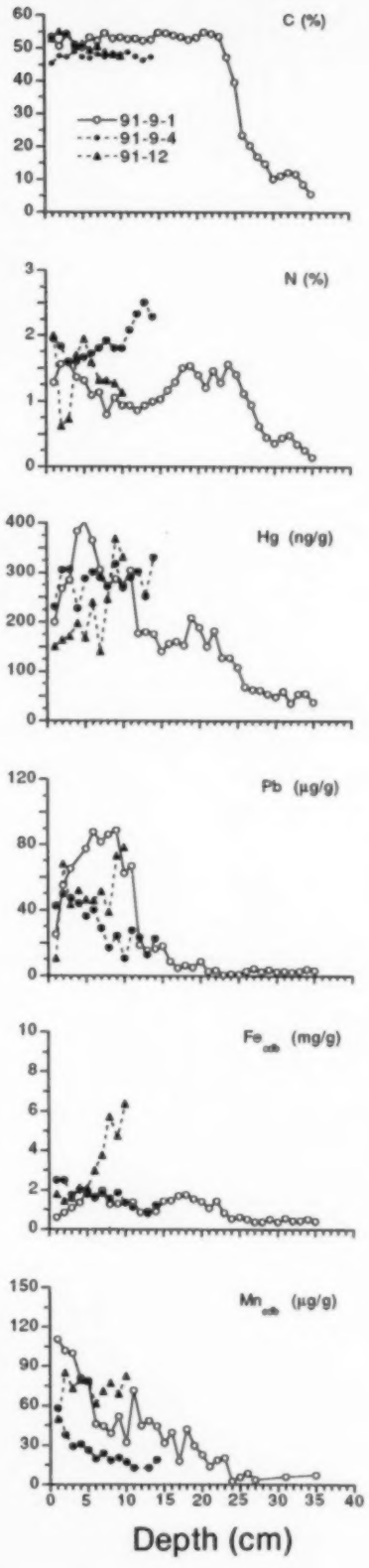


Fig. 3. C, N, Hg, Pb, Fe, and Mn profiles for unflooded (open symbols) and flooded (solid symbols) wetland soils sampled in the Cabonga watershed.

Flooded soils

The flooded podzols sampled in the LG-2 reservoir (Fig. 4*c*) display the same characteristics as the northern pristine podzols described above (Figs. 4*a* and 4*b*), having thin organic horizons containing up to 40% organic C and underlying mineral horizons that are less than 10% C. Compared with pristine soils, noticeable differences are observed only at the surface of the flooded soils. A marked drop in C appears at the surface at station 92-17 and within the first 3–4 cm at stations 92-24 and 92-101 (Fig. 4*c*). At stations 92-17 and 92-101, the lowest N levels (average 0.5%) are limited to the first centimetre and do not extend past the first 5 cm as in the pristine cores. The entire organic layer for all flooded podzols is 6 cm thick or less. The C profiles in flooded wetland soils (stations 90-20 and 91-23; Fig. 5*a*) and flooded peat soils (stations 91-9-4 and 91-12; Fig. 3; 91-21 and 92-23; Fig. 5*c*) remain comparable to their pristine equivalents. In contrast, the N profiles show a redistribution of the maximum concentrations towards the surface of the submerged cores. A marked decline in redox potential ($Eh_{Ag/AgCl}$) was observed in the organic horizon of inundated cores (<−100 mV) as compared with unflooded soils (>400 mV).

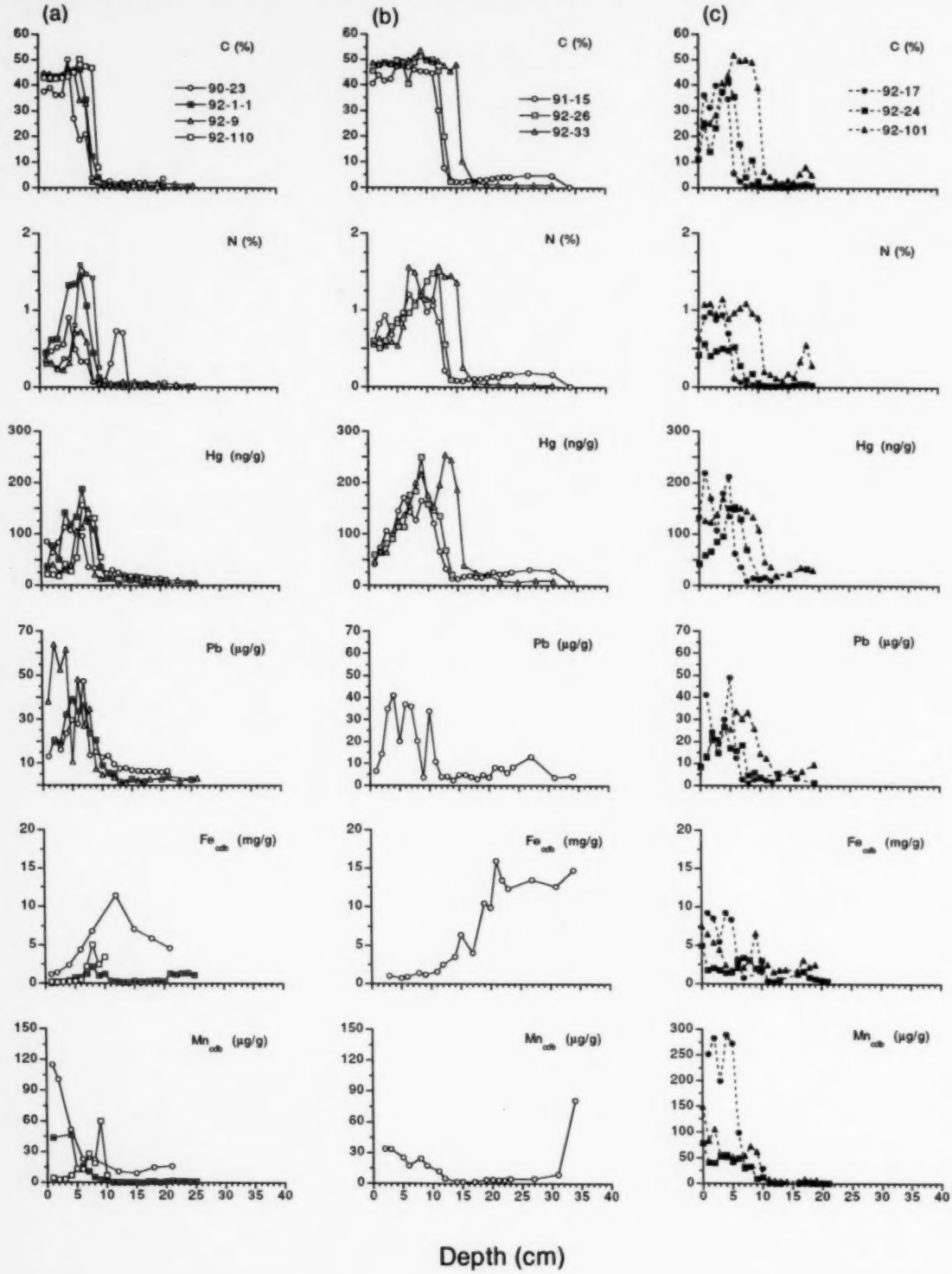
The profiles of Fe and Mn oxyhydroxides in podzols and wetland soils perturbed by inundation show generally a significant redistribution of these metals toward the sediment–water interface (Figs. 4*c* and 5*a*). Although reactive Fe and Mn are concentrated primarily in the first several centimetres of the inundated podzols, these metals extend throughout the entire organic cover of wetland soils.

Although the maximum concentrations of Hg and Pb are exclusively associated with the organic horizon of flooded podzols of the LG-2 reservoir, two stations (i.e., 92-17 and 92-101; Fig. 4*c*) exhibit elevated concentrations of Hg beginning with the uppermost centimetre and extending to a depth of 8–10 cm. Lead profiles of flooded podzols show a slight upward displacement of this metal as compared with a pristine profile. The maximum levels of heavy metals in flooded wetland soils are not found at the base of the organic cover, but extend throughout the entire core. In the flooded peat soils (Fig. 5*c*), the profiles of Hg and Pb are still uniform over the entire depth of the sampled cores; however, the maximum levels of these metals are significantly higher (*t* test; $p < 0.001$) in flooded cores (189 ± 35 $ng \cdot g^{-1}$ and 23 ± 13 $\mu g \cdot g^{-1}$, Hg and Pb, respectively) as compared with pristine cores (96 ± 24 $ng \cdot g^{-1}$ and 4 ± 5 $\mu g \cdot g^{-1}$, Hg and Pb, respectively).

Hg, Pb, and C burdens in humic horizon soils

Accurate calculation of elemental burdens in soils depends upon the determination of dry densities of the soils. For example, Lindqvist (1991) used an average dry density of 0.07 to 0.12 $g \cdot cm^{-3}$ over the entire organic horizon of Swedish podzols. Jensen and Jensen (1991) reported dry densities in the top 50 cm of Scandinavian bog soils varying between 0.015 and 0.12 $g \cdot cm^{-3}$. In the present study dry densities, evaluated at 1-cm intervals for each group of

Fig. 4. C, N, Hg, Pb, Fe, and Mn profiles for (*a*) pristine *Cladina* sp. podzols and (*b*) pristine *Pleurozium* sp. podzols sampled in the La Grande complex, and (*c*) flooded podzols sampled in the La Grande-2 reservoir.



soils, show a significant intrinsic and extrinsic heterogeneity (Table 1). This variability may, if it is not taken into consideration, bias the metal burden calculations for the soils. Hg burden (Table 2) seems to be specific to each soil type (ANOVA; $p < 0.05$). No statistical difference (t test; $p > 0.1$) is noted between pristine soil burdens (Table 2) and those of their flooded equivalents (Table 3).

Discussion

Pristine soils

Processes of incorporation of Hg and Pb in different pristine soils

The average levels of Hg and Pb measured in the organic horizons of all soils in the present study (Figs. 2–5) are comparable to those obtained for Swedish soils (250 to 350 $ng \cdot g^{-1}$ and 32 to 64 $\mu g \cdot g^{-1}$, respectively) (Aastrup et al. 1991; Johansson et al. 1991; Lindqvist 1991). The strong association of heavy metals with particulate organic matter is a common characteristic of the three groups of forest soils studied. These results are consistent with those reported in the scientific literature (Dudas and Pawluk 1976; Gotoh and Koga 1977; Friedland et al. 1984; Lodenius et al. 1987; Semu et al. 1987; Severson et al. 1992; Arakel and Hongjun 1992; Dmytriw et al. 1995). The stable hydrated Hg species, Hg(II), can be complexed by organic ligands possessing the S^- radical (Schuster 1991) and (or) with carboxyl groups of humic acids (Allard and Arsenie 1991), thus contributing to the fixation of Hg to soil organic matter.

In the organic horizon, the distribution of Hg and Pb is specific to each group of soils. Lodenius et al. (1987) have shown in an in vitro study that the behaviour of Hg varies with soil type. In podzols, the profiles of heavy metals are similar in both the Cabonga and La Grande regions. The maximum levels of Hg and Pb are confined to the area immediately below the surface, i.e., under the leaf litter of Cabonga reservoir podzols (Fig. 2) and under the moss cover (*Cladina* sp. or *Pleurozium* sp.) of the LG-2 reservoir podzols (Figs. 4*a* and 4*b*). Without considering the litter or living surface of podzols, the regression coefficients between organic C and heavy metals concentrations in the humic horizon are much higher: $r^2 = 0.93$ ($p < 0.001$) for Hg–C association and $r^2 = 0.83$ ($p < 0.001$) for Pb–C association. Dmytriw et al. (1995) indicated that Hg is preferentially associated with partially degraded organic matter of such soils. Whereas humic compounds may form solid gels having a low solubility, trace metals can penetrate the gels, accumulate in this phase, and remain stable as long as the podzol is well drained (Arakel and Hongjun 1992).

In wetland environments, where the percentage of water is higher, the metal–organic association remains stable until periods of high precipitation. Seasonal saturation of soils facilitates the dissolution of the gels, and thus encourages the temporary mobility of metals in the interstitial water (Arakel and Hongjun 1992). The organic acids with the Hg adsorbed onto them can then be transported either horizontally or vertically within the soils (Aastrup et al. 1991; Johansson et al. 1991; Lindqvist 1991; Mierle and Ingram 1991). Downward transport is clearly shown in the Hg and Pb profiles of the wetland soils of the LG-2 region (Figs. 5*a* and 5*c*). In these soils, Hg appears to be more abundant at depth than Pb. In contrast, in the single wetland soil obtained from the Cabonga region (Fig. 3), maximum concentrations of Hg and Pb remain, as with the podzols, confined to the upper several centimetres found immediately below the living moss cover. It is possible that more efficient drainage in Cabonga wetland soils may inhibit downward metal mobility as compared with soils of the La Grande region. The permanent saturation of the La Grande watershed peat soil (91-16; Fig. 5*c*) may facilitate the mobility of humid-Hg complexes in the interstices of *Sphagnum* sp. moss. It is likely that this mobility contributes to the uniformity of the Hg profile throughout the depth of the pristine core.

Elemental burdens in the humic horizons of Quebec soils

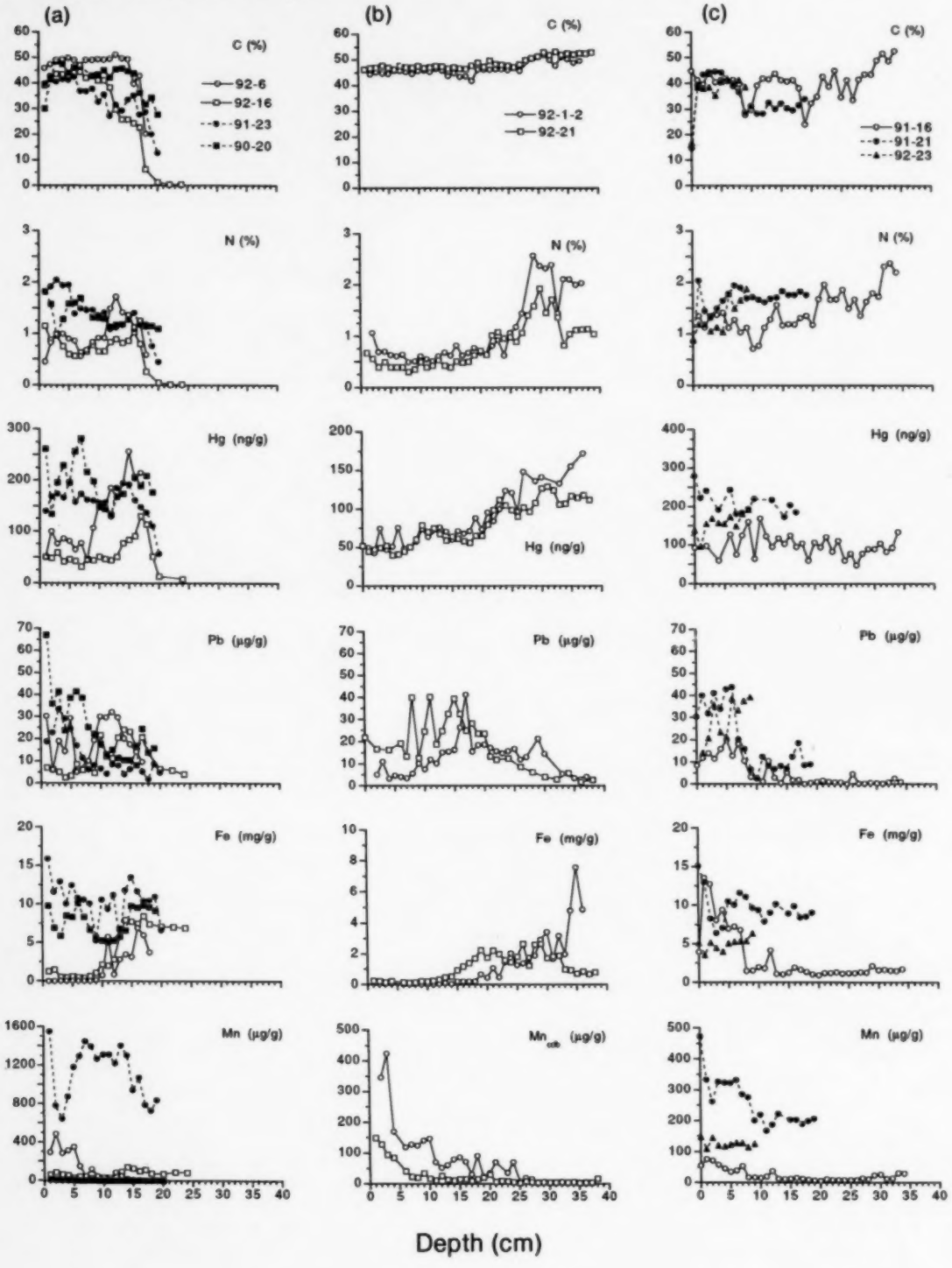
The average Hg burden measured in the organic horizon differs from one group to another (Table 2). *Cladina* sp. podzols are characterized by a low Hg burden (2544 ± 258 $\mu g \cdot m^{-2}$) compared with *Pleurozium* sp. podzols and wetland soils (average 5115 ± 877 and 6223 ± 1280 $\mu g \cdot m^{-2}$, respectively). These notable differences suggest that a fraction of the Hg is transported from upland soils toward poorly drained soils of low-lying areas. In upland areas, Aastrup et al. (1991) observed horizontal transport of Hg to the water course within the first 50 cm of forest soils. The flux of Hg reaches its climax during the wet season, when the rising water table carries the dissolved organic compounds (humic and fulvic acids) and their associated Hg to water courses (Johansson et al. 1991; Lee and Iverfeldt 1991; Lindqvist 1991; Meili et al. 1991). Up to 30% of the Hg incorporated into the soils can be transported to lakes via surficial runoff (Aastrup et al. 1991; Johansson et al. 1991; Mierle and Ingram 1991; Nater and Grigal 1992; Lucotte et al. 1995).

The average Hg burden found for pristine northern peat soils (3069 ± 643 $\mu g \cdot m^{-2}$; Table 2) is probably underestimated, as neither the mineral horizon nor bedrock was reached during sampling. We are thus unable to confirm that these soils represent Hg sinks as contended by several authors (Aastrup et al. 1991; Johansson et al. 1991; St. Louis et al. 1994). As in the case of peat soils, the number of samples obtained in the Cabonga region is too small to assure representative sampling. The leaching of Hg from well-drained soils toward low-lying areas seems, however, to be confirmed (Table 2). The thin podzols possess an average Hg burden (4100 $\mu g \cdot m^{-2}$) lower than that of either a thick podzol (7300 $\mu g \cdot m^{-2}$) or a wetland soil (11 600 $\mu g \cdot m^{-2}$).

Heavy metal burdens in the Cabonga (47°N) and LG-2 (53°N) regions

The average maximum concentrations of Hg and Pb, determined in the organic horizons of podzols in La Grande region (116 ± 58 $ng \cdot g^{-1}$ and 19 ± 12 $\mu g \cdot g^{-1}$, respectively; Figs. 4*a* and 4*b*), are two times lower (t test; $p < 0.001$) than the ones measured in the podzols of the Cabonga region (246 ± 106 $ng \cdot g^{-1}$ and 60 ± 39 $\mu g \cdot g^{-1}$, respectively; Fig. 2). An initial interpretation of this difference could be the diminution of anthropogenic fallout of these metals

Fig. 5. C, N, Hg, Pb, Fe, and Mn profiles for (*a*) unflooded (open symbols) and flooded (solid symbols) wetland soils (thin), (*b*) unflooded wetland soils (thick), and (*c*) unflooded (open symbols) and flooded (solid symbols) peat soils sampled in the La Grande complex.



at higher latitudes (i.e., over regions remote from the highly industrialized centres). However, when comparing the Hg burden of the various podzols in Quebec (Table 2), latitudinal differences do not appear as large as those in Sweden or in the United States. Swedish, low-latitude soils contain 4 to 5 times more Hg than soils at higher latitude (Johansson et al. 1991). In the north central United States, soils closer to centres of industrialization seem to accumulate up to five times as much anthropogenic Hg as soils farther west (Nater and Grigal 1992). In Quebec, the northern podzols (3646 ± 1671 $\mu g \cdot m^{-2}$) display up to 70% of the Hg burden of the southern podzols (5181 ± 1986 $\mu g \cdot m^{-2}$). This difference is not statistically significant (*t* test; $p > 0.1$) because it is smaller than the intrinsic variability of Hg burdens within this group of soils.

Anthropogenic inputs of Hg to northern Quebec, distant from industrial and urban sources, appear to be relatively uniform. Our results are similar to those of Lucotte et al. (1995) who concluded, from an investigation of natural lakes in the same regions, that Hg transported by atmospheric processes from sources of industrial contamination is deposited uniformly throughout central Quebec. This is consistent with the absence of a north/south Hg gradient such as that observed in Norway in *Sphagnum* sp. moss and bog soils (Steinnes and Andersson 1991). Gaseous Hg or Hg(II) fixed on aerosol size particles have a residence time of approximately 1 year (Nater and Grigal 1992; Slemr and Langer 1992). Because intrahemispheric mixing occurs over periods of 3 – 4 months (Slemr and Langer 1992), this metal can be transported several thousands of kilometres from its source before being deposited (Glass et al. 1986; Nater and Grigal 1992; Lucotte et al. 1995).

The pronounced decrease in the Hg burden with increase in latitude observed in Sweden can be attributed to climatic factors that contribute to the distinct ecologies and pedologies of southern and northern regions. Northern soils accumulate less Hg because their organic horizons are thinner and less dense than those of southern soils (Lindqvist 1991). By normalizing Hg burden to the organic C content of the organic horizon, the latitudinal gradient is diminished by a factor of two (Meili 1991). This reduced gradient is more consistent with results obtained in our study, where no significant gradient is observed. In contrast to the weak latitudinal gradient for Hg in the Quebec soils, we have observed that (southern) Cabonga podzols accumulate, on average, twice as much Pb (*t* test; $p < 0.003$) as La Grande podzols (1527 ± 351 and 728 ± 110 $\mu g \cdot m^{-2}$, respectively; Table 2). Our results suggest that atmospheric Pb is transported over shorter distances than Hg, probably because it tends to associate more rapidly with coarse dust particles and aerosols (Lucotte et al. 1995).

Flooded soils

In the Cabonga reservoir (67 years of impoundment), the comparison of heavy metal profiles before and after flooding is not appropriate because few representative stations could be sampled. However, such a comparison can be made in the Cabonga region where recent flooding has been caused by beaver dams, and in the LG-2 region where the reservoir was filled in 1979.

Behaviour of heavy metals in flooded podzols

The rapid decline in Eh in the first several centimetres of inundated podzols indicates considerable bacterial activity in the submerged organic horizons (Gunnison et al. 1985; Jackson 1991). When compared with their pristine equivalents (Figs. 4*a* and 4*b*), a significantly lower concentration of C is observed in the first several centimetres of the flooded cores (Fig. 4*c*). Although this loss of C could indicate a degradation of organic material, it is not clear why the decrease is always confined to the uppermost portion of the cores, and why it is much more obvious in certain cores (stations 92-24 and 92-101) than in others (station 92-17). A 50–80% degradation of initial C is unlikely, given the refractory nature of this type of organic matter (Morisson and Thérien 1991; Louchouarn et al. 1993).

The low levels of C in the surface layer of flooded podzols can be more easily attributed to recent accumulation of C-poor lacustrine-type material on top of the former soil surface. Such a surface sedimentary layer is characterized by a C:N ratio (≈34) lower than that of the pristine forest soil (C:N ≈ 81). The differing nature of the surface cover is supported by stable isotope data on surficial C from both a flooded wetland soil (station 90-20; Fig. 5*a*) and a flooded peat soil (station 91-21; Fig. 5*c*). The first several centimetres of these cores are composed of autochthonous organic material distinctly impoverished in ^{13}C (−29 to −31‰) as compared with that of the surface of pristine soils (−25 to −26‰; Chaire de recherche en environnement Hydro-Québec/CRSNG/UQAM 1992, 1993). These lower isotopic signatures were interpreted as representing a periphyton layer covering shallow soils (Louchouarn et al. 1993).

The flooded podzol (station 92-17; Fig. 4*c*), covered by a thin sedimentary layer (1 cm thick) deposited since impoundment, appears to have preserved the pre-existing humic layer with its Hg load intact. The average concentrations of C and Hg are comparable to those in pristine podzols. In contrast, at the stations where the recent sediment layer is thicker (4 cm, i.e., 92-24), the thinness of the underlying humic horizon (2–3 cm) suggests that the living *Cladina* sp. cover and a portion of the undifferentiated organic horizon has been eroded and (or), degraded, taking with it the N and associated metals (Fig. 4*c*). Louchouarn et al. (1993) have already suggested that resuspension of a portion of the organic cover of flooded soils may be one of the most efficient ways to transfer Hg from soils to the water column. A recent experiment conducted in the LG-2 reservoir to simulate resuspension of the humic horizon of flooded soils appears to confirm this hypothesis (Mucci et al. 1995). The extent of these erosion and resedimentation processes varies from soil to soil and likely depends on the soil position within the reservoir and its proximity to the drawdown zone. *Cladina* sp. is undoubtedly more vulnerable to erosion by ice, wind, and waves and is more biodegradable than *Pleurozium* sp. or *Sphagnum* sp., which are already saturated with water in natural systems.

Behaviour of heavy metals in flooded wetlands

In contrast to submerged podzols of the same region, the behaviour of Hg in wetland soils appears to be more

Table 1. Mean dry densities calculated at 1-cm intervals in various groups of pristine and flooded soils.

	Depth (cm)	Density ($\mu g \cdot cm^{-3}$)	
		Pristine	Flooded
Cladina sp. podzols	0–4	0.15	0.19
	5–7	0.38	0.34
	8–11 (<10%C)	1.43	1.43
	≥12 (<2%C)	3.05	3.05
Pleurozium sp. podzols	0–3	0.057	0.057
	4–5	0.15	0.15
	9–13	0.43	0.43
	14–15	0.61	0.61
	16–19 (<10%C)	1.43	—
	≥20 (<1%C)	3.05	3.05
Wetland soils			
Thin	0–4	0.057	0.057
	5–8	0.15	0.15
	9–13	0.29	0.29
	14–17	0.43	0.43
Thick	0–4	0.048	0.048
	5–8	0.12	0.12
	9–11	0.15	0.15
	12–18	0.20	0.20
	19–21	0.24	0.24
	22–28	0.28	
	≥29	0.32	
Peat soils	0–4	0.048	
	5–8	0.063	
	0–2		0.043
	3–5		0.062
	6–8		0.073
	9–11	0.078	0.075
	12–18	0.10	0.10
	19–21	0.12	0.12
	22–28	0.14	
	≥29	0.16	

influenced by abrupt geochemical changes provoked by inundation (Fig. 5*a*). The profiles show a redistribution of Hg, Pb, reactive Fe, and N toward the sediment–water interface. This difference in the behaviour of Hg suggests that the association of Hg with organic material may be of a different nature in wetland soils. The Hg already associated with dissolved organic material (humic and fulvic acids) in pristine wetland soils can, following inundation, easily migrate in the interstices of mosses. However, this redistribution does not seem to be instantaneous. For example, maximum concentrations of Hg are found at depth within a wetland soil recently flooded by a beaver dam in the Cabonga region (station 91-12; Fig. 3). The presence of living tamaracks implies that inundation had occurred within 1 year. In contrast, in a wetland soil obtained from a neighbouring beaver pond (station 91-9-4) where dead trees suggest an impoundment history of 1–3 years, the Hg is already redistributed within the organic horizon.

Despite the conspicuous redistribution of Hg and Pb in submerged wetland soils, the potential loss of heavy metals to the water column may be limited. Some authors contend that a chemical barrier is formed by a layer of oxyhydroxides precipitated at the sediment–water interface (Evans 1989; Dmytriw et al. 1995). This barrier traps upwardly diffusing reduced metals before they reach the water column. The behaviour of heavy metals in pristine wetland soils resembles that of pristine peat soils, already permanently saturated.

Table 2. Organic horizon depths, C, Hg, and Pb burdens, and C:N ratios calculated for the organic layer of the various groups of pristine soils sampled in the Cabonga and La Grande watersheds.

Soil group	Station	Depth (cm)	Hg burden ($\mu g \cdot m^{-2}$)	C burden ($g \cdot m^{-2}$)	Atomic C:N	Pb burden ($mg \cdot m^{-2}$)
Cabonga podzols (thin)	91-7	6	4 929	5 626	25	1933
	LU-012	4	3 332	2 688	27	1305
Cabonga podzols (thick)	90-9	9	7 281	15 362	43	1344
La Grande podzols (*Cladina* sp.)	90-23	8	2 177	7 276	77	714
	92-1-1	8	3 228	11 197	61	757
	92-9	8	2 116	9 941	136	902
	92-110	9	2 656	13 002	110	629
La Grande podzols (*Pleurozium* sp.)	91-15	12	4 042	13 466	56	639
	92-26	13	4 449	13 847	62	—
	92-33	15	6 854	18 063	51	—
Cabonga wetland soils	91-9-1	28	11 563	30 558	49	1541
La Grande wetland soils	92-6	17	6 058	17 972	61	847
	92-16	17	2 684	13 233	58	600
	92-1-2	37+	8 427+	37 313+	64	1021+
	92-21	39+	7 726+	42 406+	89	1119+
Cabonga peat soils	91-6	23+	2 500+	8 944+	66	549+
La Grande peat soils	91-16	36+	3 524+	16 406+	34	124+
	92-5	39+	2 615+	22 970+	33	—

Note: Numbers with the + symbol are probably underestimated. —, no data.

It is difficult to evaluate the influence of inundation on the redistribution of heavy metals in peat soils because their pristine equivalents show uniform distribution of these metals. Although the average concentrations of Hg in flooded peat soils are statistically higher than those obtained in nonflooded peat soils, this difference may result from the inherent heterogeneity of the peat soils that comprise the James Bay region. The entry and accumulation of Hg into these ecosystems will occur at different rates depending on their hydrologic regimes (Jensen and Jensen 1991). This regime is a complex factor to evaluate, and in consequence, the geochemical fate of Hg in these ecosystems is difficult to predict. A more detailed study is essential to fully appreciate the data generated from poorly drained areas.

Heavy metals burdens in northern soils before and after inundation

The humic horizon of *Cladina* sp. podzols represents smaller reservoirs of Hg than the thicker organic layer of wetland soils (Table 2). Following inundation, these soils can, however, influence the degree of short-term Hg contamination in hydroelectric reservoirs. Their structural vulnerability resulting from the presence of *Cladina* sp. moss and their undifferentiated and partially degraded organic matter appear to facilitate erosion by wave action (Louchouarn et al. 1993; Mucci et al. 1995). Significant losses in organic C, Hg, and Pb burdens are indeed revealed by data for the flooded podzol from station 92-24 (Table 3). In addition, *Cladina* sp. flooded podzols are an ideal habitat for burrowing insect larvae, which may be important vectors for the transfer of Hg to higher levels of the food chain (Tremblay et al. 1995). The humic horizon of the flooded podzols is also the site of the most intense Hg methylation (up to 50 $ng \cdot g^{-1}$, i.e., 14 times higher than in pristine podzols) (Mucci et al. 1995). Nevertheless, in the three flooded podzols not subjected to erosion (stations 92-8, 92-17, and 92-101), total Hg burden is not reduced after flooding (Tables 2 and 3). This strong fixation of Hg to soil organic matter may be responsible for the minimal diffusive flux of dissolved Hg observed in the water column of the LG-2 and LA-1 reservoirs (Montgomery et al. 1995).

As the structure of the organic matter composing the surface layers of wetland and peat soils is resistant to bacterial degradation, the average organic C burden remains practically unchanged following inundation of these environments (Table 3). With their thick organic horizon, the wetland soils represent the largest reservoir of Hg in the boreal forest environment (average burden ranging from 5100 to 8000 $\mu g \cdot m^{-2}$). After impoundment, the Hg and

Table 3. Organic horizon depths, C, Hg, and Pb burdens, and C:N ratios calculated for the organic layer of the various groups of flooded soils sampled in the Cabonga and La Grande watersheds.

Soil group	Station	Depth (cm)	Hg burden ($\mu g \cdot m^{-2}$)	C burden ($g \cdot m^{-2}$)	Atomic C:N	Pb burden ($mg \cdot m^{-2}$)
La Grande podzols	92-8	8	2263	—	—	—
	92-17*	5	2256	4 834	48	411
	92-24*	4	1701	4 113	89	221
	92-101*	7	2975	9 932	55	—
Cabonga wetland soils	91-12	11+	2440+	5 343+	29	559+
	91-9-4	15+	6059+	10 114+	33	581+
La Grande wetland soils	92-20*	17	7419	16 528	39	745
	91-23	19	6934	14 201	29	402
La Grande peat soils	91-21*	16+	2557+	4 298+	24	222+
	92-23	9+	827+	2 002+	35	156+
	92-38	11+	913+	—	—	—

Note: Numbers with the + symbol are probably underestimated. —, no data. *, C and Hg burdens are calculated without the lacustrine surficial layer.

Pb burdens of the flooded wetland soils remain almost intact, despite a marked redistribution of these metals towards the sediment–water interface. In the case of flooded peat soils, no significant physical changes in the Hg and Pb profiles could be detected following inundation. Nevertheless, it is impossible to estimate the efficiency of Hg and Pb retention from burden calculations (Table 3), because the base of the organic layer was not reached during sampling. The only major transformation of the Hg burden in the flooded wetland and peat soils is methylation of up to 10% of the Hg formerly present in the living cover of *Sphagnum* sp. and *Pleurozium* sp. mosses of these environments (Chaire de recherche en environnement Hydro-Québec/CRSNG/UQAM 1993).

Conclusion

Although the soils of central Quebec constitute a mosaic of distinct geochemical and ecological systems, it is possible to group them into three categories on the basis of the geochemistry of their transition metals (Fe and Mn) and heavy metals (Hg and Pb). The hydrologic regime of forest soils appears to play a determining role in the transport of dissolved organic material and its associated Hg load. Surficial runoff transports humic-Hg complexes from upland soils (podzols) towards more poorly drained areas situated at the bases of slopes. These low-lying wetland soils accumulate significant Hg burdens. In wetland soils, seasonally water-saturated, humic-Hg complexes appear to be leached downward in the organic horizon in dry season.

The present study contrasts estimates of long-distance atmospheric transport of Hg to Quebec with estimates from other countries. In northern countries, average atmospheric fluxes of this metal have been reported to be on the order of 10 to 20 $\mu g \cdot m^{-2} \cdot year^{-1}$ (Mierle 1990; Aastrup et al. 1991; Lindqvist 1991; Swain et al. 1992). Accepting that these influxes have been important for about the past 50 years (Lucotte et al. 1995), this corresponds to a total Hg input of approximately 750 $\mu g \cdot m^{-2}$ (averaging 15 $\mu g \cdot m^{-2} \cdot year^{-1}$ since the beginning of industrialization). The Hg burden in the organic horizon of each soil studied (Table 2) is considerably higher than the estimated value of 750 $\mu g \cdot m^{-2}$. There are two possible explanations for this difference (*i*) preindustrial background levels of Hg are overestimated, or (*ii*) atmospheric inputs are underestimated. If we assume a total anthropogenic input of Hg of 750 $\mu g \cdot m^{-2}$, then we must conclude that of the calculated average burden of northern Quebec soils (2500 $\mu g \cdot m^{-2}$), 70% (or ≈1750 $\mu g \cdot m^{-2}$) is of natural origin. These values are higher than those reported by Aastrup et al. (1991) who suggest that between only 17 and 31% of the Hg burden in the humic layer of Swedish podzols is of natural origin. If we assume that the Swedish values are reasonable, the estimate of atmospheric inputs of Hg to the boreal forest could easily exceed 36 $\mu g \cdot m^{-2} \cdot year^{-1}$ (70% of 2500 $\mu g \cdot m^{-2}$ over 50 years). Hg inputs could come from sources in the American midwest or from sources farther away. Direct and indirect atmospheric inputs of Hg and other trace metals may constitute a great threat to aquatic environments. It has been noted that most of the fish caught in natural boreal forest lakes have Hg concentrations that exceed the maximum permissible for sale (Lathrop et al. 1991; McMurtry 1989; Verta 1990; Lindqvist 1991; Meili 1991).

Upon inundation, soils in reservoirs support intense bacterial activity as reflected by the decrease in the Eh, the redistribution of nutrients, the production of CH_4 and CO_2, and the methylation of Hg. This activity does not represent a measurable decline in the particulate organic C from the humic horizons of the soils, which are primarily composed of refractory C. If direct release of Hg from flooded soils occurs, it is not evidenced by a marked decline

in the initial burden of Hg in the organic horizon. In contrast, significant quantities of Hg may be released to aquatic organisms living in the reservoir through erosion of the organic cover of *Cladina* sp. podzols. This study suggests that the initial reserves of Hg in the LG-2 reservoir have been only slightly depleted after 11 to 13 years of impoundment. The potential for contamination of aquatic organisms living in the reservoir could therefore remain high for a considerable length of time.

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Mercury remobilization from flooded soils in a hydroelectric reservoir of northern Quebec, La Grande-2: results of a soil resuspension experiment[1]

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Abstract: An experiment was conducted during summer, 1992, in a protected bay of the La Grande-2 reservoir (northern Quebec) to evaluate the role of erosional processes in the transfer of mercury from a flooded soil to the water column. Up to 57% of the organic carbon, approximately 4% of the inorganic mercury, and nearly 71% of the methylmercury from the humic horizon of a flooded podzol were transferred to the water column and transported outside the experimental perimeter in the form of particulate matter after one resuspension event. In contrast, total dissolved mercury concentrations in the water column decreased during the resuspension event as a result of scavenging by suspended particles. We postulate that most of the methylmercury produced in the submerged soils is associated with fine-grained organic particles that remain in suspension for some time before flocculating and accumulating at the sediment–water interface. We suspect that these particles are a potential food source and can be readily ingested by pelagic and benthic detritus-feeders as well as trapped by periphyton. Resuspension and erosion processes along the exposed shores of a recently impounded reservoir may thus accelerate the transfer of mercury, primarily as methylmercury, from flooded soils to predatory fish.

Résumé : Une expérience fut exécutée durant l'été 1992 dans une baie protégée du réservoir La Grande-2 dans le moyen-nord québécois afin d'évaluer le rôle des processus d'érosion dans le transfert du mercure d'un sol inondé vers la colonne d'eau. Jusqu'à 57% du carbone organique, environ 4% du mercure inorganique, et près de 71% du méthyl-mercure contenu dans l'horizon humique d'un podzol ennoyé ont été transférés dans la colonne d'eau et transportés hors du périmètre expérimental sous forme de matière particulaire après un seul épisode de resuspension. En contrepartie, la concentration de mercure total dissous dans la colonne d'eau a diminué durant l'épisode de resuspension suite à son adsorption par les particules en suspension. Nous postulons que la plupart du méthyl-mercure produit dans les sols ennoyés est associée à de fines particules de matière organique qui demeurent en suspension pendant quelque temps avant de flocculer à l'interface eau–sédiment. Ces particules peuvent servir de nourriture et sont facilement assimilées par les organismes détritivores pélagiques et benthiques en plus d'être interceptées par le périphyton. Les processus d'érosion et la resuspension le long des rives exposées d'un réservoir récemment mis en eau pourraient donc accélérer le transfert du mercure, et tout particulièrement du méthyl-mercure, des sols ennoyés vers les poissons prédateurs.

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Introduction

Currently, the main cause of mercury (Hg) contamination in fish caught in hydroelectric reservoirs is believed to be the remobilization of Hg from inundated soils to the water column following microbial degradation of the terrestrial organic matter (Abernathy and Cumbie 1977; Bodaly and Hecky 1979; Cox et al. 1979; Jackson 1988*a*, 1988*b*) and the microbially mediated formation of methylmercury (CH_3Hg^+) (Furutani and Rudd 1980; Bodaly et al. 1984; Jackson 1988*a*, 1991; Hecky et al. 1991; Morrison and Thérien 1991), the most toxic and abundant form of Hg in fish. Most of the Hg accumulated in soils is of anthropogenic origin (Nriagu 1989; Grondin et al. 1995). The Hg has been transported from point sources over long

distances through the atmosphere and distributed uniformly over land and sea by wet and dry deposition (Bjorklund et al. 1984; Verta et al. 1989; Slemr and Langer 1992; Lucotte et al. 1995).

In a recent publication (Louchouarn et al. 1993), we proposed that, following impoundment of a reservoir, erosion of the humic or O-horizon (A_0- + A_1-horizons; Wilde 1958) of the soil profile by drawdown, wind-induced waves, and ice scouring may accelerate the transfer of Hg to aquatic biota. Several authors have already noted that sediment resuspension is a very efficient remobilization mechanism for Hg in a variety of natural aquatic environments, including lakes (e.g., Hulscher et al. 1992), rivers (e.g., Jackson et al. 1982), and marine estuaries (Lindberg and Harris 1977; So 1980).

Our current hypothesis is based on a number of recent, well documented observations. First, in forest soil profiles, high Hg concentrations are confined to the O-horizon, suggesting a strong association between Hg and organic matter. The strong affinity of Hg for partially degraded organic material was confirmed by selective extraction and analysis of soil components (Dmytriw et al. 1995). Between 60 and 80% of the total Hg contained in the O-horizon of both a pristine (unflooded) and a submerged soil and up to 85% of that found in a lake sediment was isolated following its extraction with organic matter by a 1 M sodium hydroxide (NaOH) solution. Second, the high organic carbon (C) and nitrogen (N) content of the flooded soils at La Grande-2 (LG-2) after 14 years of impoundment suggests that the microbial degradation of terrestrial organic matter is a slow process at northern latitudes. Likewise, the organic horizon of a submerged soil, unaffected by erosion, remains enriched in Hg, indicating that chemical remobilization of the metal to the overlying waters does not deplete its Hg burden significantly (Grondin et al. 1995). Finally, complete erosion of the soil profile can be observed along the exposed shores of the LG-2 reservoir and, in some areas, has been noted to extend to depths reaching nearly 4 m, and as much as 10 m in areas affected by drawdown.

To verify our hypothesis that erosion of the soil profiles may promote the transfer of Hg to aquatic biota, we designed and conducted a field experiment during which the humic horizon of a flooded soil in a well protected bay was artificially resuspended. Several geochemical characteristics of the water column and sediment were measured before, during, and after the resuspension event.

Materials and methods

Study area

The experiment was conducted in a semi-enclosed, protected bay of the LG-2 reservoir (53°32′N, 77°27′W; Fig. 1) where the soil profiles have remained relatively undisturbed since impoundment and the influence of drawdown has been negligible. LG-2 is part of the hydroelectric complex on the La Grande River near James Bay, a region located on the granitic bedrock of the Precambrian Canadian Shield. Reservoir impoundment occurred over a 1-year period between 1978 and 1979. The bay is subdivided into two arms (north and south). The south arm is limited in its extent by a dike (D-24). The inundated soils are mainly podzols covered with *Sphagnum* spp. moss, *Cladonia* spp., black spruce (*Picea mariana*), grey pine (*Pinus banksiana*), and Labrador tea (*Ledum groenlandicum*). A detailed description of the soil types can be found elsewhere (Grondin et al. 1995).

A number of sampling sites in the north and south arms of the bay were selected and marked with flagging tape to ensure that the same sites were visited repeatedly (Fig. 1). In August 1992, when the resuspension experiment was carried out, water temperatures ranged from 16.3°C at the surface down to 9°C beneath a broad seasonal thermocline located at a depth of 3–4 m at the deepest sites sampled in the north (site 22, 6 m) and south (site 18, 6.75 m) arms of the bay. Similarly, dissolved oxygen (O_2) concentrations decreased from 10 mg/L at the surface to 1.2 mg/L at the deepest stations. The pH at the surface was variable but always within the range of 5.8 to 6.7.

Resuspension

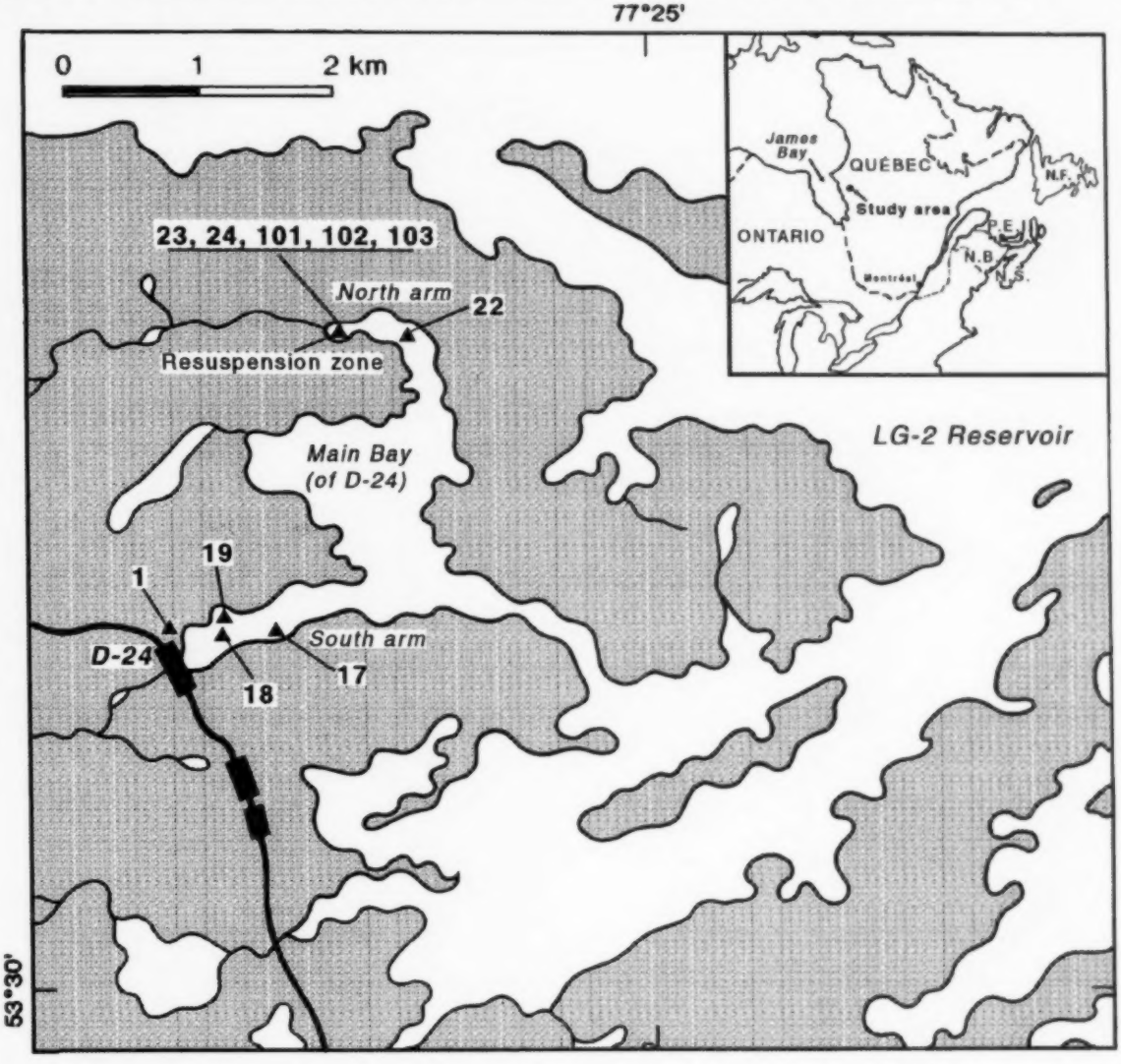
The organic horizon of flooded soils along the shores of a relatively confined area of the north arm of the bay (sites 23, 24, 101, 102, 103 in Fig. 1) was resuspended over a period of 3 days from August 3 to 5, 1992 (days of year 215 to 217). The experimental resuspension area had a surface area of approximately 250 m^2 and a maximum depth of 2 m. The water column appeared to be well mixed over this depth range. Thirteen people participated in the experiment. Within the resuspension perimeter, where water depths rarely exceeded 1.5 m, small trees and shrubs were uprooted with shovels, rakes, and cutters, thereby releasing particulate matter into the water column. Larger, partially submerged trees with extensive root systems were completely uprooted with the aid of a manual winch.

Sample collection

The water column was sampled before, during, and after resuspension for analysis of dissolved Hg and particulate matter both within and outside the resuspension perimeter as well as in the south arm of the bay. Flooded soils were also collected at the same sites before and after the resuspension. Water samples were taken from an aluminum boat anchored on shore or from submerged trees. Water column temperature and O_2 profiles were obtained using a YSI model 57 probe before each sampling. Given the homogeneity of the water column within the resuspension perimeter, all samples were collected at only one depth, 0.8 to 1.0 m below the surface.

All sampling equipment and containers were rigorously cleaned in a cold 10% nitric acid (HNO_3) solution for 24 h and rinsed several times with NANOpure® deionized distilled water. Sixty-millilitre Teflon bottles were capped and double sealed in acid-washed Ziploc® plastic bags. All other sampling equipment was stored in resealable plastic bags. Water was pumped from depth using a manually operated peristaltic pump, a section of Masterflex silicone tubing, Teflon sampling hose at the inlet, and a Millipore 47-mm in-line filter holder assembly at the outlet. The latter held two filters, a glass fiber (GF) filter and a 0.45-μm GN-6 Gelman filter. The former was used as a prefilter to prevent rapid clogging of the latter. The sampling hose, weighted at the end, was lowered to the desired

Fig. 1. Map of the experimental area in the LG-2 hydroelectric reservoir of the James Bay area of northern Quebec.



sampling depth. Water was pumped through to rinse the tubing, the filter assembly, and the Teflon bottles before they were filled and repackaged. The bottles were kept in a cooler on ice until they were returned to the field laboratory where they were acidified to pH 2 with Ultrapure® hydrochloric acid (HCl) prior to analysis. Water samples were flown back to Montréal where total dissolved Hg analyses were performed within 36 h of sampling. Details of the sampling procedures are found elsewhere (Montgomery et al. 1995).

Suspended particulate matter (SPM) was separated from approximately 150 L of water collected using a battery-operated pump and stored in precleaned 50-L polyethylene carboys. The particulate fraction was isolated by continuous-flow, temperature-controlled centrifugation at 10 000 rpm or 8500 × *g*. Known volumes of water were also filtered on preweighed GF and 0.45-μm membrane filters to determine the SPM concentration. The centrifugation extracts were transferred to Teflon bottles and the filters were stored in acid-cleaned Petri dishes. Both were kept frozen until analysis in Montréal.

Unfortunately, not enough material could be collected on the filters before clogging to obtain reliable measurements of total Hg and CH_3Hg^+ content. Thus, these were determined on SPM isolated by centrifugation. Preliminary experiments indicate that the centrifuge extracts 90 and 72–85% of the mass of SPM collected by the GF and 0.45-μm filters, respectively. Given that the smallest and lightest particles were not retained by the centrifuge, it is likely that concentrations of total Hg and CH_3Hg^+ in SPM

reported in this study are minimal estimates. Smaller particles offer the most surface area per unit weight and thus are more likely to be characterized by higher concentrations of these species.

Undisturbed cores of the flooded soils were collected by divers using 15-cm diameter polyvinyl chloride (PVC) tubes. The cores were sectioned at 1-cm intervals over their entire length (up to 20 cm) as they were sequentially extruded from the tube. Subsamples of the solids from each interval were placed in 30-mL preweighed plastic vials for water content determination and the remainder of each section was sealed in a plastic bag. All sediment samples were kept frozen until analysis.

Laboratory analyses

Water column samples were pretreated prior to analysis to release and oxidize Hg associated with dissolved organic matter (DOC) (Goulden and Anthony 1980). Fifty microlitres of a saturated potassium persulphate solution was added to a 5-mL aliquot of the sample in a precleaned quartz tube. The tube was sealed with Parafilm®, and the mixture was mixed thoroughly and subjected to ultraviolet oxidation for 20 min. The total dissolved Hg concentration of the treated samples was determined by cold vapour atomic fluorescence spectrometry (CV-AFS) using a modification of the method of Bloom and Fitzgerald (1988) described elsewhere (Montgomery et al. 1995). Briefly, 4 mL of the irradiated solution was injected into a reactor where Hg(II) is reduced to Hg(0) by a stannous chloride solution. The elemental Hg(0) was carried in a precleaned and water-saturated stream of argon (Ar) gas directly to a quartz cell where its fluorescence was measured. The detection limit (signal to noise ratio = 3) of this method is approximately 0.2 pg of Hg (0.1 ng/L for a 4-mL sample) with a precision of better than 5% between 10 and 100 pg (for details see Montgomery et al. 1995).

Total Hg in the flooded soils and SPM were analyzed by CV-AFS following acid digestion of a weighed amount of freeze-dried or wet solid (~0.1 g dry weight) in 10 mL of a 10:1 mixture of concentrated HNO_3 and 6 M quartz-distilled HCl (Agemian and Chau 1976). The digestion was carried out in Pyrex® test tubes (25 mm o.d. × 250 mm long) held at 120°C for 6 h in an aluminum block. The upper part of the test tubes was cooled by forced air to induce condensation. This method was described previously (Louchouarn et al. 1993). In the case of wet samples, results were converted to a dry weight basis using water content determined by freeze-drying of separate aliquots. Tests showed that there was no noticeable loss of Hg or CH_3Hg^+ as a result of freeze-drying. The reproducibility and accuracy of this method were evaluated at ±7% on the basis of 80 measurements of a certified sediment standard, National Research Council Canada BEST-1.

CH_3Hg^+ concentrations in the flooded soils and sediments were determined by procedures described by Ma (1994). Briefly, CH_3Hg^+ was released from 0.1–0.2 g of freeze-dried sediment by 4 mL of an acid cupric bromide solution (Horvat et al. 1988). The methylmercury bromide (CH_3HgBr) is isolated from the mixture by three consecutive extractions with 5 mL of toluene. The extracts are combined and the toluene is partially evaporated by air-flow to a weighed final volume of between 0.5 and 1.0 mL. Fifty microlitres of this concentrated solution is injected onto a chromatographic column (12 × 1/8 in. i.d., (1 in. = 25.4 mm) 10% free fatty acid phase on 80/100 Chromosorb W AW, Supelco) and CH_3Hg^+ eluted at 150°C in a 60 mL/min Ar flow. The eluate is thermally decomposed to Hg(0) in a heated quartz wool tube at >500°C, and quantitatively measured by CV-AFS. The detection limits are in the order of 0.2 pg or 1 ppb with recovery efficiencies averaging 86% (± 18%) on standards that were run with each batch of 5–10 samples (n = 30).

This method, however, could not be used on the SPM because substances co-extracted with CH_3HgBr appeared to interfere with the performance of the chromatographic column. Alternatively, CH_3Hg^+ was isolated by distillation (Horvat et al. 1988). A weighed fraction of the SPM (1–10 mg) was mixed in a small distillation tube with 0.7 mL of water, 0.1 mL of a 4 M potassium bromide (KBr) solution, and 0.2 mL of 2 M sulphuric acid (H_2SO_4). The distillation was carried out at 85°C in an Ar flow (~40 mL/min), and the distillate was recovered in 1 mL of pure water cooled in an ice-water bath. The Hg content was measured by CV-AFS before and after persulphate – UV oxidation, as described above. The CH_3Hg^+ concentrations were calculated from the difference between the two values. Recovery from the distillation was determined on standards processed with each batch of eight samples and averaged 82% (± 13%, n = 20).

Finally, the total C and N concentrations in the soil and SPM samples were determined with a Carlo-Erba elemental analyzer. Reproducibility and precision of these measurements were in the order of 1%. As carbonates were nearly absent from the soil profile (<0.06% C, dry weight; Dmytriw et al. 1995), total C was essentially equivalent to organic C.

Results and discussion

Soil profiles

The distributions of organic C, lead, Hg, and other trace metals in typical pristine podzol profiles are described in detail by Grondin et al. (1995) and Dmytriw et al. (1995). Briefly, the soils have a thin organic-rich horizon, 5–15 cm deep, and are characterized by a sharp transition to the mineral zone below which little organic C can be found (Fig. 2A). Hg concentrations are highest in the O-horizon, reaching maximum values of up to 250 ng/g (ppb) or 1.25 pmol/g (dry weight) in the partially degraded material found below recognizable needle and twig debris. They decrease abruptly at the bottom of the organic zone to less than 40 ppb. CH_3Hg^+ in these soils can only be detected in the organic-rich zone. Its concentration in the O-horizon is typically in the order of 4–5 ng/g (Chaire de recherche en environnement 1993).

Upon flooding, podzols undergo a number of physical and geochemical changes: the displacement of air by water, the consumption of pore-water O_2, and the migration of the oxic–suboxic boundary towards the newly established sediment–water interface (Grondin et al. 1995; Dmytriw et al. 1995). Nevertheless, the C distribution in a flooded soil remains similar to that of a dry forest soil (Figs. 2A

Fig. 2. Organic C, total Hg, and CH_3Hg^+ distribution in (A) dry podzol from the experimental area (site 1, core 92-1-1D) and (B) submerged podzol before resuspension (site 101, core 92B-101-1A) profiles from within the experimental perimeter at LG-2.

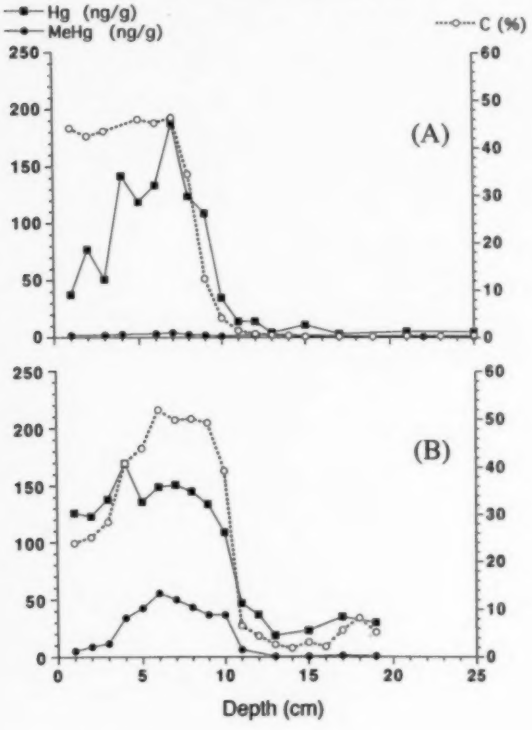
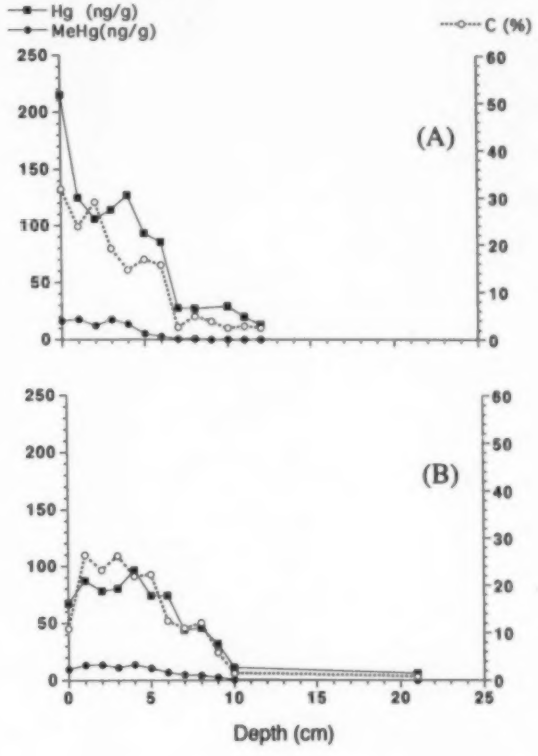


Fig. 3. Organic C, total Hg, and CH_3Hg^+ distribution in two inundated podzol profiles from within the experimental perimeter at LG-2 after the resuspension event ((A) site 101, core 92B-101-1A; (B) site 103, core 92B-103-1A).



and 2B) with the exception of a notable decrease in concentration in the top 5 cm. This latter observation could be explained by a degradation of the organic matter near the sediment–water interface or the accumulation of autochthonous sedimentary material (Louchouarn et al. 1993), but most likely by the winnowing by wind-induced waves in this shallow-water environment. The ^{13}C signature of the organic matter near the surface indicates that it is still mostly of terrestrial origin but has been slightly modified by partial degradation, differential winnowing, and colonization by aquatic plants and microfauna (Chaire de recherche en environnement 1993).

The integrated total Hg burden of the O-horizon of flooded podzols is generally unaffected by the impoundment (Grondin et al. 1995), although a redistribution of Hg appears to have occurred at site 101 (Figs. 2A and 2B). The apparent surface enrichment is believed to result from the accumulation of resuspended Hg-rich refractory organic material that contributes little to the integrated burden because of its high water content. CH_3Hg^+, however, is much more abundant in the flooded soil than in its unflooded counterpart. In fact, very little if any CH_3Hg^+ is ever detected in a dry soil profile (Chaire de recherche en environnement 1993). CH_3Hg^+ is synthesized by bacteria and accumulates under the suboxic conditions (Jackson 1988*a*; Driscoll et al. 1994) that are established within the flooded soils as a result of labile organic matter degradation. In general, CH_3Hg^+ concentration profiles in flooded podzols nearly parallel the distribution of organic C. Maximum concentrations, however, are found between 5 and 10 cm below the sediment–water interface. The position of this peak supports the hypothesis put forth above that resuspended refractory organic material probably accumulated at the sediment–water interface after flooding because Hg methylation will be promoted by the degradation of labile soil organic matter buried below. Values as high as 22 and 55 ng Hg/g were measured in the O-horizon of flooded podzols from both arms of the bay (Chaire de recherche en environnement 1993), making up nearly 30% of the total Hg burden of the humic horizon of the submerged soils (Table 1).

Four days after the end of the resuspension event, several cores were collected within the experimental perimeter (Fig. 3). Examination of the profiles reveals that most, if not all, of the O-horizon was resuspended, while part of the mineral zone, composed of fine sands, was disturbed. The new organic-rich horizon was thinner than in the original profile, and sorting of the settling material had apparently occurred. Organic C and total Hg concen-

Table 1. Average concentrations and integrated burden of organic C and total, methyl, and inorganic Hg in the organic and mineral horizons of a podzol from site 101 sampled before and after the resuspension experiment.

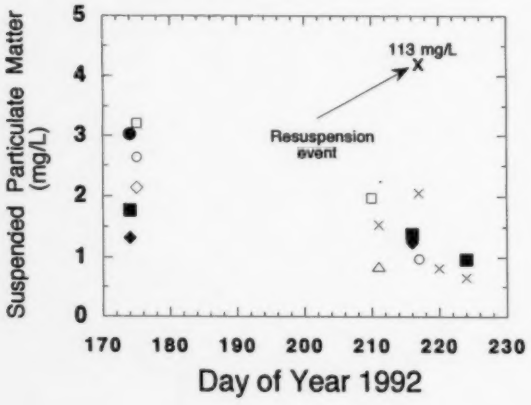
	Depth (cm)	% C	Total Hg (ng/g dry)	CH_3Hg^+ (ng/g dry)	Inorganic Hg (ng/g dry)	Dry density[a] (g/cm^3)
Before	0–4	26	129	8.5	121	0.10
	4–11	46	142	43	99	0.34
	11–19	4	32	1.9	30	1.89
After	0–6	22	124	12	112	0.40
	7–13	3	23	0.4	22.6	0.4

Integrated burden in the O-horizon

	Depth (cm)	C (g/m^2)	Total Hg ($\mu g/cm^3$)	CH_3Hg^+ ($\mu g/cm^2$)	Inorganic Hg ($\mu g/cm^2$)
Before	0–10	11 828	3750	1051	2699
After	0–6	5 135	2890	306	2584

[a]From Grondin et al. (1995).

Fig. 4. Suspended particulate matter concentrations in the water column at a number of sites in the north (open symbols) and south (closed symbols) arms of the experimental bay during the first two months of the ice-free season and which includes the resuspension event. ● (site 17), submerged podzol covered mostly by cladonia; ■ (site18), former lake sediments; ◆ (site 19), submerged peat bog; ○ (site 22), former lake sediments; □ (site 23), submerged peat bog; ◇ (site 24), submerged podzol covered mostly by cladonia; × (site 101), submerged podzol covered mostly by sphagnum moss in the resuspension perimeter; and △ (site 102), submerged podzol covered mostly by sphagnum moss.



trations were highest near the sediment–water interface and decreased progressively with depth to the mineral horizon. CH_3Hg^+ profiles still paralleled the distribution of organic C but a significant fraction of both components seemed to have been lost to the water column and (or) sedimented elsewhere.

This last observation can be confirmed by calculating the integrated burden of organic C, total and inorganic Hg, and CH_3Hg^+ in the O-horizon at site 101 before (Fig. 2A) and after (Fig. 3A) resuspension. To proceed with these calculations, however, it is necessary to assume that the O-horizons of flooded soils within the experimental perimeter have the same thickness and composition, and that the resuspended mineral particles settle back rapidly. In fact, calculations show that none of the C, total Hg, and CH_3Hg^+ was lost from the mineral horizon after the resuspension event. Results of the calculations (Table 1), however, indicate that up to 57% of the organic C, approximately 4% of the inorganic Hg, and nearly 71% of the CH_3Hg^+ were lost from the O-horizon following resuspension. Despite the uncertainties in these calculations, the results can be interpreted as indicating a selective loss of CH_3Hg^+ and organic C from the flooded soils. Furthermore, they suggest that CH_3Hg^+ and the microbial population that produces it are intimately associated with fine-grained and readily resuspended organic-rich particles. These particles would not settle out of the water column rapidly but would be transported away from the site of resuspension. This CH_3Hg^+-rich material can be ingested by pelagic suspension-feeders and benthic detritus-feeding invertebrates and (or) retained by periphyton and thus serve as a strong vector of the metal to predatory fish (Meili 1991; Parkman and Meili 1993).

Suspended particulate matter

SPM concentrations as well as their organic C, total Hg, and CH_3Hg^+ content were monitored episodically at various sites in the bay throughout the ice-free season (4 months), including the resuspension event. With the exception of the resuspension event, SPM concentrations never exceeded 3.5 mg/L and decreased significantly ($\alpha = 0.05$) throughout the sampling period (Fig. 4), irrespective of the underlying substrate. The higher concentrations found in late

Fig. 5. (A) Total Hg concentration, and (B) CH_3Hg^+ concentration of suspended particulate matter collected by centrifugation in the water column at a number of sites in the north and south arms of the experimental bay throughout the ice-free season. Symbols are as in Fig. 4.

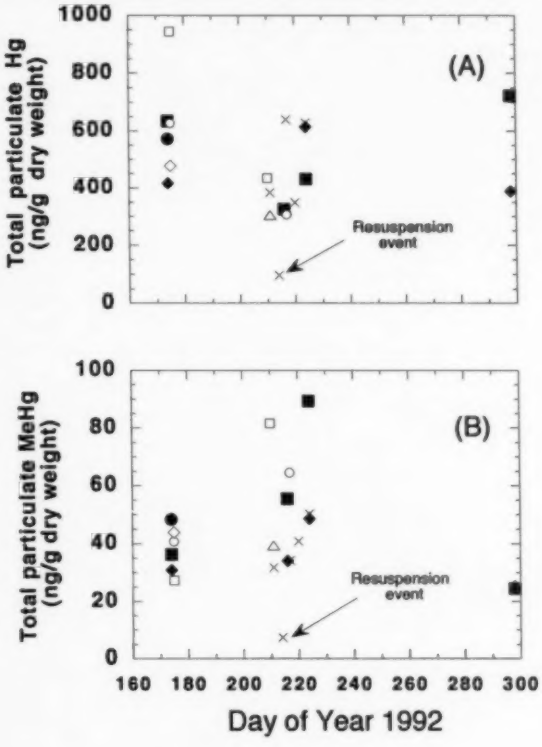
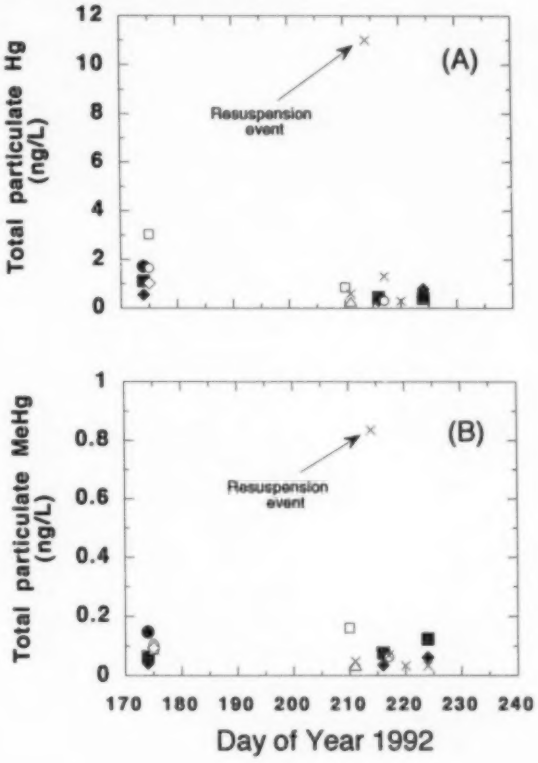


Fig. 6. (A) Total (dissolved and particulate) Hg and (B) total CH_3Hg^+ concentrations per unit volume in the water column at a number of sites in the north and south arms of the experimental bay during the first 2 months of the ice-free season, which includes the resuspension event. Symbols are as in Fig. 4.



spring most likely resulted from strong snowmelt runoff and soil erosion by ice scouring, winds, and drawdown during this part of the year. On the third day of the resuspension, the SPM concentration in the experimental perimeter reached 113 mg/L.

The organic C content of the SPM remained relatively constant at each site throughout the sampling period, varying between 25 and 36% dry weight (average 30 ± 3, n = 19, data not shown). It decreased drastically to a value of 12% immediately following the resuspension event. Similarly, the organic N content of the SPM decreased from an average of 3.3 (± 1, n = 19, data not shown) to 0.6% dry weight as a result of the resuspension event. These observations confirm that part of the mineral horizon, in addition to the O-horizon, was resuspended during the experiment, resulting in a dilution of the SPM with a mineral component. Furthermore, the nature of the organic matter in suspension was affected. Its C:N molar ratio increased from an average of 11 ± 1.5 (n = 17) to 22 (data not shown). During the resuspension event, the organic component of the SPM was dominated by partly decomposed terrestrial material (C:N >> 15; Matson and Brinson 1990).

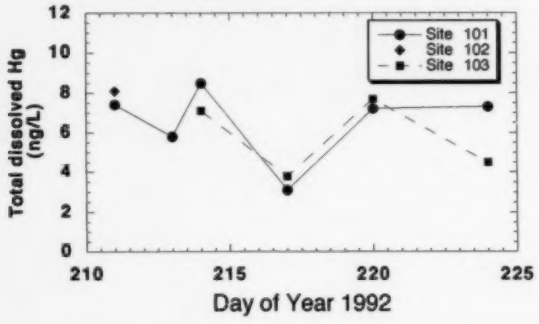
The total Hg and CH_3Hg^+ content of the SPM also decreased noticeably in comparison to the average concentrations in the unperturbed system (Figs. 5A and 5B). The impact of the resuspension event on the transfer of particulate total Hg and CH_3Hg^+ to the water column is most obvious when the concentrations of these species in suspension are presented per unit volume of water (Figs. 6A and 6B). The total Hg and CH_3Hg^+ burdens in the water column were increased 11- and 19-fold, respectively.

Dissolved Hg in the water column

The total dissolved Hg in the water column was measured a few days before, during, and after the resuspension event at three sites within the experimental perimeter. With the exception of measurements carried out during the resuspension event, the average water column concentration of dissolved Hg in the bay was 7 ± 1 ng/L (35 ± 5 pM, n = 9) (Fig. 7, see caption for comment). It decreased to half this value (3.5 ± 0.4 ng/L or 17 ± 2 pM, n = 2) at the two sites where the water column was sampled on the third day after the resuspension event. These results are interpreted as indicating scavenging of dissolved Hg by particulate material resuspended during the experiment.

It is well established that dissolved Hg has a strong affinity for suspended particulate matter in the water

Fig. 7. Total dissolved Hg concentrations at three sites within the experimental perimeter prior to, during, and after the resuspension event. Error bars correspond to the standard error of multiple analyses ($2 \leq n \leq 4$) on the same sample. When no error bar appears, the error is smaller than the symbol. It should, however, be noted that absolute concentrations reported here may be tainted by contamination during transport to Montréal. Variations between samples taken at various times are certainly real and conclusions drawn from these data remain valid. Later analyses, such as those reported by Montgomery et al. (1995), were carried out in a field laboratory and do not suffer from this problem.



column (Cranston and Buckley 1972; So 1980; Frenet 1981; Nishimura and Kumagai 1983; Ferrara et al. 1986, 1989). In fact, Ferrara et al. (1991) proposed that aqueous transport of dissolved Hg in a naturally contaminated environment may be of little or no importance and that the SPM carries most of the load. Furthermore, the resuspension of sediments has been proposed as a means of reducing the Hg concentration in a contaminated river system (Rudd and Turner 1983).

Partition coefficients, K_D, were calculated from the total Hg and CH_3Hg^+ concentrations associated with the SPM and the water column according to

$$K_D = \frac{[Hg]_{SPM}\ (ng/g)}{[Hg]_{diss}\ (ng/mL)}$$

The average partition coefficients for total Hg vary between 7.2 (± 3.6) × 10^4 and 2.8 (± 0.3) × 10^4, before or after and during the resuspension event, respectively. For CH_3Hg^+ ($[CH_3Hg^+]_{diss}$ = 0.3 ng Hg/L; Chaire de recherche en environnement 1993), the values are 1.5 (± 0.6) × 10^5 and 2.5 × 10^4, respectively, for the same sampling periods. Similar values were reported by Hurley et al. (1993) for total Hg (log K_D from 4.5 to 5.7) in the water column of a seepage lake in northern Wisconsin. They also noted that K_D values have been shown to decrease with increasing concentrations of sorbate, which is in agreement with results presented in this study. Furthermore, these values attest to the great affinity of both total Hg and CH_3Hg^+ for solid substrates.

The amount of particulate Hg in the water column is dependent upon the size, nature, and concentration of the particles that are held in suspension. Smaller particles will usually carry more Hg per unit weight than larger ones (Airey and Jones 1982), given their greater relative surface area. Organic-rich particles, because of their greater affinity for Hg, will generally have higher Hg concentrations than suspended clays or sands (Rae and Aston 1982).

The production and transfer of CH_3Hg^+ to fish in freshwater reservoirs

The degree of Hg contamination in fish from freshwater reservoirs has been shown to be proportional to the flooded surface area or extent of flooding (Abernathy and Cumbie 1977; Lodenius et al. 1983; Bodaly et al. 1984; Jackson 1991). Although one might assume that this would be reflected in higher concentrations of Hg in the water column, recent observations revealed that dissolved Hg concentrations in low DOC reservoir waters are similar to those measured in clear natural lakes (Montgomery et al. 1995).

Generally, more than 85% of the Hg accumulated in fish is in the form of CH_3Hg^+ (Westöö 1966; Lansens et al. 1991; Bloom 1992). A strong correlation was reported between fish Hg and CH_3Hg^+ concentrations in the water column (Gill and Bruland 1990; Driscoll et al. 1994). The detailed processes by which this contaminant is produced and transferred to the fish, however, remain a subject of much controversy. Results of this study clearly indicate that CH_3Hg^+ is produced in the O-horizon of flooded soils where inorganic Hg deposited by eolian processes before inundation (Bloom and Watras 1989; Winfrey and Rudd 1990; Lindqvist 1991; Schroeder et al. 1991; Vandal et al. 1991; Fitzgerald et al. 1991) has accumulated and is strongly bound to organic matter (Figs. 2 and 3) (Dmytriw et al. 1995). Although reliable high resolution profiles of pore-water CH_3Hg^+ in flooded soils have not yet been obtained, profiles measured in a highly contaminated estuarine marine sediment show that the diffusion of dissolved CH_3Hg^+ to the overlying water column is strongly inhibited by a thin oxic layer at the sediment–water interface (Gagnon et al. 1996). Oxic conditions or oxidized sediment components in this layer may either catalyze demethylation or decrease methylation rates (Jackson 1989; Gagnon et al. 1996).

Several authors have demonstrated that the concentration of CH_3Hg^+ in natural sediments is determined by the ratio of methylation and demethylation rates and that this ratio is strongly temperature dependent (Wright and Hamilton 1982; Callister and Winfrey 1986; Bodaly et al. 1993). As a result, it is assumed that >90% of the methylating activity in Canadian Shield lakes occurs in the epilimnetic sediments (Bodaly et al. 1993; Ramlal et al. 1993). This may explain the very high CH_3Hg^+ : total Hg ratio measured in the humic horizon of the submerged podzol (site 101) of the shallow-water bay of the LG-2 reservoir. Nevertheless, given that the epilimnion is well mixed and oxygenated, the presence of a thin oxidized layer at the sediment–water interface would hinder the release of dissolved CH_3Hg^+ to the overlying waters. The CH_3Hg^+ can, however, be transferred to the water column and the biota if it is physically resuspended with particulate matter or carried by burrowing organisms (e.g., Saouter et al. 1993; Tremblay et al. 1995). On the basis of the above arguments and results, we suspect that, in hydroelectric reservoirs, a major

portion of the Hg must be accumulated by pelagic and benthic invertebrates directly from an abiotic source, such as suspended particulate matter and sediments, and transferred up the food chain (Potter et al. 1975; Jackson 1988*b*). The Hg can be ingested directly either as CH_3Hg^+ produced in flooded soils or as inorganic Hg adsorbed onto SPM that could be methylated in either the water column or the organisms themselves (Rudd et al. 1980).

Invertebrates are probably among the most important links in the contamination of the aquatic food chain as a whole. Detrital terrigenous organic matter is a food source utilized by many invertebrates. It is characterized by higher Hg levels but lower nutritional value than primary producers. Consequently, to satisfy their needs, the invertebrates must ingest a large quantity of detrital material, which results in higher levels of contamination in detritus-feeding invertebrates than in predatory invertebrates (Andre et al. 1991; Watras and Bloom 1992; Parkman and Meili 1993).

It should be noted, however, that indigenous sediments and submerged soils are not the only source of either particulate Hg or CH_3Hg^+ in freshwater reservoirs. Surface runoff from the watershed can supply large amounts of suspended and dissolved material, including Hg-rich terrestrial organic matter, to a reservoir (Lee and Hultberg 1990; Mierle 1990; Lee and Iverfeldt 1991; St. Louis et al. 1994; Lucotte et al. 1995). These processes, however, are also active in natural lakes in which fish populations do not necessarily display the acute contamination observed in newly impounded reservoirs. Factors such as mean depth, water residence time, and water chemistry (e.g., pH, DOC-water colour), as well as other physiographic characteristics of the impounded reservoir and its watershed, will also affect how and how much Hg will be transferred to aquatic organisms (e.g., Cope et al. 1990; Bloom et al. 1991; Jackson 1991; Johnson et al. 1991; Mierle and Ingram 1991; Xu and Allard 1991; Miskimmin et al. 1992).

The temporal evolution of fish contamination in the LG-2 hydroelectric reservoir

Verdon et al. (1991) observed that Hg levels in the flesh of lake whitefish, *Coregonus clupeaformis*, increased nearly fourfold within 5 years of the impoundment at LG-2. Since then, levels have remained relatively stable while those of carnivorous fish such as northern pike, *Esox lucius*, keep increasing steadily (James Bay Mercury Committee 1993). From our observations and the results of this and previous studies, we postulate that the rapid increase in Hg levels in noncarnivorous fish results from an upsurge in microbial, methylating activity promoted by the inundation of labile terrestrial organic matter and accelerated transfer of Hg, including CH_3Hg^+, to the aquatic biota by erosion and resuspension of soils along the exposed banks of the newly impounded reservoir. As the shores of the reservoir stabilized after a few years, other processes, such as the emergence of insect larvae from shallow areas protected from erosion (Tremblay et al. 1995) and recycling at the sediment–water interface (Hurley et al. 1993), became significant in the transfer of Hg to the aquatic biota and contributed to the maintenance of relatively high levels of contamination in noncarnivorous fish. Given the relationship established by other researchers between lake size, epilimnetic temperature, and methylation activity (Wright and Hamilton 1982; Bodaly et al. 1993; Ramlal et al. 1993), we would expect that protected shallow bays such as the one in which we conducted the resuspension experiment are characterized by high methylation rates during the summer. Protected shallow bays, which are most often preferred spawning grounds for various species of fish, may be a major source of CH_3Hg^+ to the reservoir biota long after the first few years of impoundment.

Conclusions

CH_3Hg^+ makes up nearly 30% of the total Hg found in the humic horizon of a submerged podzol sampled in a shallow-water, protected bay of the LG-2 reservoir, whereas it is barely detectable in a soil of similar characteristics on land. The formation of CH_3Hg^+ in the epilimnetic, flooded soil is promoted by the degradation of labile terrestrial organic matter under suboxic conditions and relatively high summer water column temperatures.

We estimate that up to 57% of the organic C, approximately 4% of the inorganic Hg, and nearly 71% of the CH_3Hg^+ from the humic horizon of the flooded soil were transferred to the water column and deposited outside the perimeter following the resuspension experiment. Most of the exported material was probably in the form of fine-grained organic-rich particles, some of which can be assimilated by pelagic and benthic detritus feeders. Immediately following the resuspension episode, the total Hg and CH_3Hg^+ burdens of the water column were increased 11- and 19-fold, respectively. In contrast, the total dissolved Hg concentration in the water column decreased nearly twofold as a result of scavenging by the increased SPM load. Results of this experiment emphasize the importance of soil resuspension in the rapid transfer of Hg, including CH_3Hg^+, to aquatic biota during the first years of reservoir impoundment.

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PERSPECTIVE

Food-web regulation by a planktivore: exploring the generality of the trophic cascade hypothesis

Roy A. Stein, Dennis R. DeVries, and John M. Dettmers

Abstract: The trophic cascade hypothesis currently being tested in north temperate systems may not apply to open-water communities in lower latitude U.S. reservoirs. These reservoir communities differ dramatically from northern lakes in that an open-water omnivore, gizzard shad (*Dorosoma cepedianum*), often occurs in abundance. Neither controlled by fish predators (owing to high fecundity and low vulnerability) nor by their zooplankton prey (following the midsummer zooplankton decline, gizzard shad consume detritus and phytoplankton), gizzard shad regulate community composition rather than being regulated by top-down or bottom-up forces. In experiments across a range of spatial scales (enclosures, 1–9 m^2; ponds, 4–5 ha; and reservoirs, 50–100 ha), we evaluated the generality of the trophic cascade hypothesis by assessing its conceptual strength in reservoir food webs. We reviewed the role of gizzard shad in controlling zooplankton populations and hence recruitment of bluegill, *Lepomis macrochirus* (via exploitative competition for zooplankton), and largemouth bass, *Micropterus salmoides* (by reducing their bluegill prey). Reservoir fish communities, owing to the presence of gizzard shad, appear to be regulated more by complex weblike interactions among species than by the more chainlike interactions characteristic of the trophic cascade.

Résumé : L'hypothèse de la « cascade trophique », qu'on examine actuellement dans les systèmes des eaux tempérées du nord, pourrait ne pas s'appliquer aux communautés des eaux ouvertes des réservoirs, sous les basses latitudes, aux États-Unis. Dans ces réservoirs, la situation est remarquablement différente, car un omnivore des eaux ouvertes, l'alose à gésier (*Dorosoma cepedianum*), y abonde souvent. N'étant limitée ni par des poissons prédateurs (grâce à son taux élevé de fécondité et à sa faible vulnérabilité) ni par la disponibilité de ses proies zooplanctoniques (après la baisse du zooplancton au milieu de l'été, l'alose à gésier consomme des détritus et du phytoplancton), cette alose régit la composition de la communauté au lieu d'être soumise à des forces s'exerçant de haut en bas ou de bas en haut de la chaîne. Dans des expériences menées à des échelles spatiales diverses (enclos, 1–9 m^2; étangs, 4–5 ha; réservoirs, 50–100 ha), nous avons évalué la possibilité d'une application générale de l'hypothèse de la cascade trophique en mesurant sa force conceptuelle dans les réseaux trophiques des réservoirs. Nous avons examiné le rôle de l'alose à gésier dans la régulation des populations zooplanctoniques et donc dans le recrutement du crapet arlequin, *Lepomis marcochirus* (par compétition dans l'exploitation du zooplancton), et de l'achigan à grande bouche, *Micropterus salmoides* (par réduction du nombre des crapets arlequins qui leur servent de proies). Les communautés ichtyennes des réservoirs, à cause de la présence de l'alose à gésier, semblent davantage régies par un réseau d'interactions complexes entre espèces que par les interactions en chaîne caractéristiques de la cascade trophique.

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Introduction

The trophic cascade hypothesis provides a conceptual framework for examining processes underlying community structure (i.e., species composition and relative abundance of species) in freshwater ecosystems. At the heart of this concept is the premise that community structure is determined by a series of chainlike interactions between adjacent trophic levels driven by strong interactors (Kitchell and Carpenter 1993). For example, predation by piscivorous fishes reduces planktivore abundance, allowing herbivorous zooplankton to increase; as zooplankton increases, phytoplankton abundance and primary productivity decline, regardless of nutrient availability (Hairston et al. 1966; Carpenter et al. 1985; Carpenter and Kitchell 1988; Northcote 1988; for a similar perspective in stream communities, see Power 1990). Because control in this instance occurs from the top of the trophic pyramid, it is classified as top-down. In contrast, system structure can be regulated by nutrients (i.e., bottom-up control), as might be expected in eutrophic systems (see McQueen 1990; McQueen et al. 1986, 1989 for examples). However, it is likely that community regulation is not solely due to top-down or bottom-up forces (Hunter and Price 1992); instead, community composition derives from a combination of top-down (piscivore control) and bottom-up (nutrient control) forces (McQueen et al. 1986, 1989; Bartell et al. 1988; Jassby et al. 1990).

The trophic cascade hypothesis has been explored in systems ranging from relatively small-scale enclosure–exclosure experiments (e.g., Bergquist and Carpenter 1986; Post and McQueen 1987; McQueen and Post 1988; Mazumder et al. 1988, 1990; Qin and Threlkeld 1990) to whole-lake manipulations (Henrikson et al. 1980; Shapiro and Wright 1984; Kitchell and Crowder 1986; Scavia et al. 1986; Carpenter et al. 1987; McQueen et al. 1989; Vanni et al. 1990). Whole-lake tests of this hypothesis, involving manipulation of piscivores (Kitchell and Crowder 1986; Scavia et al. 1986; Carpenter et al. 1987; McQueen et al. 1989) and planktivores (Henrikson et al. 1980; Shapiro and Wright 1984; Carpenter et al. 1987; Vanni et al. 1990), have been relatively successful in predicting qualitative changes in primary production resulting from changes in upper trophic levels, particularly when manipulations have been substantial and sustained (reviewed in Carpenter and Kitchell 1988; see also Kitchell and Carpenter 1987; McQueen 1990). However, whole-lake tests have been conducted in north temperate lakes (Lake Lilla Stockelidsvatten, Sweden, Henrikson et al. 1980; Round Lake, Minnesota, Shapiro and Wright 1984; Lake Michigan, Kitchell and Crowder 1986; Scavia et al. 1986; Peter, Paul, and Tuesday lakes, Michigan, Carpenter et al. 1987; Lake St. George, Ontario, McQueen et al. 1989; Lake Mendota, Wisconsin, Vanni et al. 1990); therefore, the extension of this hypothesis to reservoirs at lower latitudes, where adjacent trophic levels do not necessarily regulate one another, may be inappropriate.

Indeed, the trophic cascade hypothesis has been demonstrated to work in some systems, but not in others (Shapiro 1990; Carpenter and Kitchell 1992; DeMelo et al. 1992; Kitchell 1992). Under what conditions does this paradigm accurately predict the outcome of trophic interactions? Specifically, researchers have pursued such questions as (*i*) is this phenomenon restricted to lakes of a particular trophic status (McQueen et al. 1989; Carney 1990), (*ii*) is it restricted to lakes and streams with depauperate communities (Strong 1992), (*iii*) is it restricted to systems with functionally significant linkages and (or) with few omnivores (Power 1992), (*iv*) is it restricted to systems that exhibit, from a variety of points of view, homogeneity (Hunter and Price 1992; Strong 1992), or (*v*) might it be limited in systems with deep-bodied, large planktivores (Hambright et al. 1991)? In this paper, we explore our ability to generalize the trophic cascade paradigm by applying concepts developed in north temperate lakes to lower latitude reservoirs.

Reservoirs versus natural lakes

Reservoirs supported more fishing activity (40%) within the United States in 1980 than any other type of freshwater ecosystem (Fisher et al. 1986). Reservoir fishing translates to 5.4 billion U.S. dollars in expenditures, yet these systems are poorly understood and managed (Hall and Harris 1986). Geographically, most U.S. reservoirs are concentrated between 30 and 42°N latitude, in nonglaciated regions, whereas natural lakes typically occur north of 42°N in glaciated regions (Thornton 1990). Thus, U.S. reservoirs tend to be warmer and shallower with longer growing seasons than natural lakes. Owing to soils in the region, southern reservoirs typically have higher total dissolved and suspended solids than natural lakes (Thornton 1990). Because reservoirs typically are located in river valleys at the base of the drainage basin (Wetzel 1990), reservoirs have larger watershed to surface area ratios than lakes, leading to high, but irregularly pulsed, nutrient and sediment inputs. Reservoirs experience dramatic, irregular water-level fluctuations that derive from a combination of flood inflows, land-use practices unfavorable for water retention, and hydropower withdrawals. With higher turbidity and greater water-level fluctuations, fewer littoral macrophytes occur in reservoirs than in north temperate lakes (Wetzel 1990). As a consequence, most primary production in reservoirs typically derives from phytoplankton, rather than from macrophytes (Kimmel et al. 1990). These structural differences between reservoirs and lakes are confounded by longitudinal gradients in physical and chemical variables between upstream, lotic areas and downstream, lentic areas of reservoirs (Thornton 1990).

Reservoirs differ from natural lakes more fundamentally. North temperate lakes, where ecological research has concentrated, are 10 000 – 12 000 yr old and formed by Pleistocene glacial activity, whereas reservoirs typically are less than 60 yr old (Noble 1986; Thornton et al. 1990). Thus, biotic interactions among fish communities in north temperate lakes typically have been shaped by evolution (MacLean and Magnuson 1977), whereas those within reservoirs have not. Rather, reservoir fish communities are comprised of species with little history of co-occurrence (Noble 1986), resulting in unique, complex interactions without representation in well-studied natural lakes. Paleoecological records from lakes suggest long periods of trophic equilibrium; similar periods of stasis do not occur in reservoirs (Kimmel and Groeger 1986).

Species introductions characterize reservoirs. Assembled fish communities include predators such as largemouth bass (*Micropterus salmoides*), walleye (*Stizostedion vitreum*), crappie (*Pomoxis* spp.), and channel catfish (*Ictalurus punctatus*). Prey species typically include bluegill (*Lepomis macrochirus*), other sunfishes (*Lepomis* spp.), golden shiners (*Notemigonus crysoleucas*), fathead minnows (*Pimephales promelas*), gizzard shad (*Dorosoma cepedianum*), and threadfin shad (*Dorosoma petenense*). Of these, gizzard and threadfin shad are most common, often accounting for 30% or more of all fish biomass within communities (Jenkins 1957, 1967; Grinstead et al. 1976; Timmons et al. 1978; Ploskey and Jenkins 1982; Johnson et al. 1988). These two species provide most prey for predators (Jenkins 1967; Timmons et al. 1978; Johnson et al. 1988) and typically have been those prey species most often introduced into reservoirs as additional prey for predatory sport fishes (DeVries and Stein 1990). Dominance of reservoir fish communities by gizzard shad is, in our view, the single most important difference between lakes and reservoirs with respect to the application of the trophic cascade hypothesis. To set the stage for our argument, we first describe how gizzard shad become dominant in reservoirs and then describe how this species (one not common to north temperate systems; Trautman 1981), from its position within the food web, acts to influence trophic levels above and below it.

Gizzard shad success in reservoirs

The relatively small size of zooplankton in reservoirs compared with north temperate lakes may predispose reservoirs to successful recruitment of gizzard shad (Bremigan and Stein 1994). Larval gizzard shad have smaller mouth gapes than bluegill or walleye in spite of the fact that they all depend on plankton immediately post-hatching (Bremigan and Stein 1994; J.B. Mion, Aquatic Ecology Laboratory, Department of Zoology, The Ohio State University, Columbus, OH 43212-1194, unpublished data). In turn, larval gizzard shad prefer smaller zooplankton (0.5–0.7 mm) than bluegill (Bremigan and Stein 1994) and may grow faster on small rather than large zooplankton. After gizzard shad drive zooplankton to low densities by intense planktivory (DeVries and Stein 1992; Dettmers and Stein 1992), they switch to feed on benthic, detrital ooze of eutrophic reservoirs (Mundahl 1991), likely never succumbing to the consequences of zooplankton overgrazing. Hence, successful gizzard shad recruitment in reservoirs may be driven by the presence of small zooplankton, combined with abundant detritus rich in organic material.

Gizzard shad are preferred over sunfishes as prey (Lewis and Helms 1964; Aggus 1973), at times contributing >90% of the diets of largemouth bass (Johnson et al. 1988; Carline et al. 1987). Yet, owing to fast growth that permits young-of-the-year gizzard shad to exceed predator gape (Hambright et al. 1991), only about 20% of gizzard shad mortality is due to predation by typical resident predators (Carline et al. 1984; Johnson et al. 1988).

Thus, neither resources (bottom-up forces) nor predators (top-down forces) control gizzard shad populations. What then prevents gizzard shad from completely dominating the fish community in reservoirs? We suggest the answer is episodic winterkill of gizzard shad. Atypically long winters lead to subsequent gizzard shad mortality (Adams et al. 1982*a*) and may reset the fish community by reducing adult gizzard shad abundance and resultant interspecific competitive effects.

Interactions between gizzard shad and zooplankton

Strictly planktivorous early in life, gizzard shad become omnivorous after reaching 25–35 mm total length (TL) and feed on zooplankton, phytoplankton, and detritus (Tiffany 1921; Dendy 1946; Kutkuhn 1957; Miller 1960; Pahl and Maurer 1962; Bodola 1966; Cramer and Marzolf 1970; Jester and Jensen 1972; Jude 1973; Mayhew 1977; Barger and Kilambi 1980; Drenner et al. 1986; Mundahl 1988). With these ontogenetic diet shifts, gizzard shad successively feed at different trophic levels (i.e., zooplankton and phytoplankton) (Tiffany 1921; Kutkuhn 1957; Miller 1960; Bodola 1966; Jester and Jensen 1972; Heidinger 1983; Todd and Willis 1985; Mundahl 1988; DeVries and Stein 1992). As omnivores, gizzard shad are not regulated by the primary consumers, i.e., zooplankton (even though they exert strong control over this level), unlike occupants of the intermediate level in north temperate systems (e.g., dace, *Phoxinus* spp., Carpenter et al. 1987; cisco, *Coregonus artedii*, Vanni et al. 1990).

As with other open-water planktivores (Brooks and Dodson 1965; Lazzaro 1987), gizzard shad can influence the abundance and species composition of zooplankton communities. When threadfin shad were stocked into a Kansas reservoir, large zooplankton were eliminated much earlier in the year than before shad stocking (Prophet 1982, 1985, 1988). Zooplankton density was negatively associated with threadfin shad abundance in two Arizona lakes (Ziebell et al. 1986) and elsewhere (Cramer and Marzolf 1970; Applegate and Mullan 1971; Cashman 1988; Kissick 1988).

Though most fishes spawn in spring and produce zooplanktivorous larvae, gizzard shad exceed the impact of these fishes, simply because of their extremely high fecundities (approaching 500 000 eggs/female, Bodola 1966; Wilder and Vondracek 1988; Parrish and Vondracek 1989), which can, in some years, translate into high larval fish densities (up to 86 fish/m^3, DeVries and Stein 1992) that can exert strong predation pressure on zooplankton. Negative relationships have occurred between abundance of larval gizzard shad and crustacean zooplankton in two Ohio reservoirs. During 1986–1989 zooplankton abundance declined within 1–2 wk of peak larval gizzard shad abundance in Kokosing Lake, Ohio, and within 2 wk of peak larval threadfin shad abundance in Stonelick Lake, Ohio (DeVries et al. 1991; DeVries and Stein 1992). To determine if juvenile gizzard shad were responsible for these zooplankton declines, we conducted an in-lake enclosure experiment where young gizzard shad were either present or excluded. In enclosures with gizzard shad (at typical reservoir densities), zooplankton densities fell to zero within 2 wk; without gizzard shad, zooplankton densities remained above 500/L (DeVries and Stein 1992). These findings have been reinforced by a consumption model for young-of-the-year gizzard shad (Dettmers and Stein 1992).

Because gizzard shad suppress crustacean zooplankton, their presence in reservoirs will strongly influence recruitment (i.e., survival through critical early life stages) of fishes with obligate zooplanktivorous larvae. For example, bluegill larvae migrate from the littoral zone to the limnetic zone immediately after hatching, where they feed on zooplankton for 4–8 wk until reaching 20–25 mm TL, at which point they migrate back to the littoral zone (Faber 1967; Werner 1967, 1969; Amundrud et al. 1974; Keast 1980; Beard 1982; Werner and Hall 1988; DeVries et al. 1991). Larval crappie also make extensive use of open-water plankton (Mayhew 1977; Overman et al. 1980; Hanson and Qadri 1984; D.R. DeVries, M.T. Bremigan, and R.A. Stein, unpublished data). Thus, competitive interactions among gizzard shad and other limnetic larvae (such as bluegill and crappie) could dramatically influence growth, survival, and ultimate recruitment of these fishes.

Effects of gizzard shad on phytoplankton

The trophic cascade hypothesis applied to reservoirs predicts that in the presence of gizzard shad, crustacean zooplankton abundance should be reduced and, as a consequence, phytoplankton abundance should increase. However, in addition to their direct effects on zooplankton, gizzard shad can directly and indirectly influence phytoplankton communities. Adult gizzard shad can reduce large phytoplankton >60 μm (e.g., *Ceratium* spp.; Drenner et al. 1984) and permit small forms (1–4 μm) to increase by enhancing the turnover rate of small algae via nutrient recycling (Threlkeld and Drenner 1987; Lazzaro et al. 1992), with the size of phytoplankton removed being inversely related to fish size (Drenner et al. 1984; Mummert and Drenner 1986). In our gizzard shad enclosure–exclosure experiment (described above), we found that edible phytoplankton (primarily *Cryptomonas* spp.) declined in enclosures with gizzard shad relative to fishless enclosures (DeVries and Stein 1992). These data, combined with diet data identifying phytoplankton in gizzard shad diets (Kutkuhn 1957; Miller 1960; Bodola 1966; Baker and Schmitz 1971; Heidinger 1983; Drenner et al. 1984; Todd and Willis 1985; Mundahl 1988), suggest that gizzard shad directly influence phytoplankton, complicating predictions from the trophic cascade hypothesis. In addition, nutrient uptake may increase through the summer (Kitchell et al. 1975; Johnson et al. 1988; D.R. DeVries and R.A. Stein, unpublished data), as young-of-the-year gizzard shad grow.

To summarize, gizzard shad are not controlled by zooplankton abundance (i.e., bottom-up control does not appear to operate). Rather, gizzard shad modify community composition of both zooplankton (directly via predation) and phytoplankton (indirectly through zooplankton and directly via herbivory and nutrient uptake) communities.

Complex effects of gizzard shad on fish communities

Gizzard shad can influence reservoir communities in a complex fashion, mediated through several trophic levels. First, because annual variation in the density and spawning time of gizzard shad is substantial, their vulnerability to predators may vary across years. When gizzard shad spawn late, their young-of-the-year are vulnerable to young-of-the-year largemouth bass for much of the summer (see Johnson et al. 1988 for a discussion of this phenomenon in Ohio reservoirs). However, when gizzard shad spawn early, they grow beyond the morphological capability of young-of-the-year largemouth bass to consume them (see general review in Adams and DeAngelis 1987). As a result, growth of young-of-the-year largemouth bass declines, for they are forced to consume less preferred foods. Thus, the size of young-of-the-year largemouth bass at the end of the summer growing season varies greatly as a result of these size-structured interactions. This variability in young-of-the-year size has dramatic implications for largemouth bass recruitment; small bass have been postulated to die at a disproportionately higher rate during winter than large individuals (Aggus and Elliott 1975; Shelton et al. 1979; Timmons et al. 1980; Adams et al. 1982*a*; Gutreuter and Anderson 1985), likely because of depletion of lipid reserves in smaller fish (Saiki and Tash 1978; Oliver et al. 1979; Adams et al. 1982*b*; Ludsin 1994). Hence, gizzard shad may well control recruitment success of largemouth bass in reservoirs by influencing size of young-of-the-year largemouth bass and ultimately their overwinter survival. Reduced recruitment of largemouth bass can further enhance the ability of gizzard shad to succeed, leading to their domination of the system.

Both gizzard shad and bluegill have limnetic zooplanktivorous larvae, but gizzard shad typically spawn 1–3 wk before bluegill; thus, larval gizzard shad precede larval bluegill in the limnetic zone. Because young-of-the-year gizzard shad can drive zooplankton to low levels, we hypothesize that reduced zooplankton abundance deprives bluegill of zooplankton, reducing bluegill recruitment. In fact, in Kokosing Lake, Ohio, abundance of larval gizzard shad is inversely related to abundance of littoral young-of-the-year bluegill (DeVries and Stein 1992).

Normally, if bluegill survive and grow in the limnetic zone, they migrate inshore at about 20 mm (Werner 1967; Werner and Hall 1988), where they serve as prey for young-of-the-year largemouth bass. If gizzard shad reduce survival of limnetic larval bluegill such that few bluegill migrate to the littoral zone, then prey available to young-of-the-year largemouth bass (which remain in the littoral zone as young-of-the-year) is reduced and bass growth declines. Data collected from two lakes in Ohio, before and after introduction of threadfin shad, provide insight into this perspective (note that as larvae and juveniles <30 mm TL, threadfin shad and gizzard shad are both obligate zooplanktivores). After being stocked into both lakes, threadfin shad had little impact on the zooplankton in Clark Lake, whereas in Stonelick Lake, threadfin shad eliminated the zooplankton at a time when larval bluegill required it as a food source. Hence, in Clark Lake, we observed what we would consider an excellent environment for growth and survival of larval bluegill, whereas in Stonelick Lake less than ideal conditions existed. If bluegill larvae recruiting to the littoral zone are important to young-of-the-year largemouth bass, then we expect higher bass growth (post-threadfin shad) in Clark than in Stonelick lake. This is precisely what happened. Largemouth

Table 1. Predicted year-class strength of bluegill and largemouth bass in Ohio reservoirs as dictated by gizzard shad abundance, zooplankton productivity, and relative spawning time of gizzard shad and bluegill.

		Scenario I	Scenario II
Gizzard shad abundance	Zooplankton productivity	Bluegill and largemouth bass year-class strength Excellent → Poor	Bluegill and largemouth bass year-class strength Excellent → Poor
Absent	High		
Low	High		
High	High		
Absent	Low		
Low	Low		
High	Low		

Note: Under scenario I, young-of-the-year gizzard shad appear in the limnetic zone before bluegill, whereas under scenario II gizzard shad appear in the limnetic zone after bluegill.

bass growth was not depressed in Clark Lake in the post-threadfin shad year, though we did not document fish in largemouth bass diets. However, in Stonelick Lake, largemouth bass growth was depressed substantially in the post- versus pre-threadfin shad year (DeVries et al. 1991). Herein, we have compared between years and lakes for both threadfin and gizzard shad across different predator densities. Consequently, the scenario of shad controlling zooplankton abundance, eventual bluegill recruitment to the littoral zone, and diet and growth of young-of-the-year largemouth bass is likely, but certainly not the whole story (witness Clark Lake).

Testing food-web regulation by gizzard shad

If our hypothesized interactions are correct, then we expect differential year-class success of bluegill and largemouth bass in reservoirs with differing gizzard shad density and zooplankton productivities (Table 1). Without gizzard shad, bluegill and largemouth bass should experience variable year-class success. If larval gizzard shad occur in the limnetic zone before larval bluegill, then bluegill and hence largemouth bass should exhibit intermediate to poor year-class strength, regardless of gizzard shad abundance and zooplankton productivity. If young-of-the-year gizzard shad appear in the limnetic zone after larval bluegill, then bluegill and bass should exhibit excellent to poor (with high zooplankton productivity) or intermediate to poor (with low zooplankton productivity; Table 1) year-class strength. Even if larval gizzard shad occur in the limnetic zone after larval bluegill, the presence of larval shad will overlap with bluegill larvae, thereby at least partially reducing bluegill recruitment success. As is perhaps obvious, we predict that gizzard shad absence is a necessary, but not sufficient, condition for excellent recruitment of bluegill and largemouth bass. However, when this keystone species is present, the opportunity for successful recruitment of sport fishes is greatly limited.

These predictions can be tested explicitly by randomly sampling adult bluegill and largemouth bass from reservoirs for which we have historical information, including temporal patterns of larval fish abundance and zooplankton productivity. By aging individuals of these two species, we can determine the occurrence and relative frequency of strong and weak year-classes, permitting us to judge the validity of these predictions (Table 1).

Exploring generality across systems

As Hunter and Price (1992) suggest, species at any trophic level can drive community dynamics. With gizzard shad, we have an excellent example of a species within the food web influencing not only trophic levels below it, but also above it. Clearly, eutrophic reservoirs that support gizzard shad differ from those aquatic systems described by Oksanen et al. (1981), where, as productivity increases, the food web shifts from three to four trophic levels. In their model, piscivores increase in abundance as productivity increases from very low levels (Persson et al. 1992). In reservoirs, though, piscivores occur at low abundance and do not exert strong top-down effects. Thus, a pattern best characterized by a three-level food chain develops, with planktivores and producers limited by resources, and zooplankton strongly suppressed by planktivores.

Why do the systems described by Persson et al. (1992) support more piscivores with increasing nutrients, whereas eutrophic reservoirs do not support high densities of piscivores? In reservoirs, gizzard shad can persist at high densities even with reduced zooplankton resources. Such persistence of gizzard shad, in the face of seemingly limited resources, strengthens their top-down effects and differs from the predictions of Oksanen et al. (1981), where consumer effects are limited by low prey abundance. Power (1992) characterizes such a food chain shift as a spillover because gizzard shad exerts even greater top-down effects on zooplankton by virtue of its capacity to use alternative food resources, yet can continue to consume zooplankton.

A trophic level may not be functionally significant if it does not influence the abundance of the trophic level immediately below it (Power 1992). In our view, gizzard shad effectively neutralize the potential piscivore effect

of largemouth bass via size-structured interactions among largemouth bass, bluegill, and gizzard shad as we have described. Similar food web studies in Sweden indicate that piscivore abundance declines in more productive lakes because young-of-the-year piscivores cannot successfully compete for zooplankton resources with the resident planktivore (roach, *Rutilis rutilis*; Persson et al. 1991). Similarly, largemouth bass are not sufficiently abundant to consume many gizzard shad because of the recruitment bottleneck described above, and we may consider piscivores a functionally nonsignificant trophic level in reservoirs. Thus, we conclude that reservoir food webs are more properly described as three-level chains because the piscivore level is not functional because of reduced piscivore recruitment and the increased effect of gizzard shad on zooplankton as a result of its omnivory.

Though we have focussed on the response of one type of system to one species of fish, regulation of the type that emanates from the middle of the food web occurs not just in reservoirs containing gizzard shad, but also in those ecosystems where species within the food web enjoy both a predatory refuge (via size, habitat structure, spatial separation, etc.) and an opportunistic foraging mode. Interestingly, invaders (whether human-assisted or natural) often possess these characteristics for they establish themselves in the face of predatory pressure and typically are opportunistic in their reliance on resources.

The rusty crayfish (*Orconectes rusticus*), a species that has successfully invaded northern Wisconsin lakes (Capelli 1982) and Ohio streams (Mather and Stein 1993), is relatively invulnerable to predatory fishes (Garvey et al. 1994) and has a broad generalist diet (Hill et al. 1993; Lodge et al. 1994). They exert dramatic impacts on both upper and lower trophic levels of north temperate aquatic ecosystems, indicating that from their position from within the food web, they regulate the benthic community (Lodge et al. 1994).

Another example derives from deep, oligotrophic lakes, where freshwater opossum shrimp, *Mysis* spp., may regulate community structure via their position in the middle of the food web. As with gizzard shad, *Mysis* spp. can alter zooplankton taxonomic composition and abundance (Lasenby et al. 1986; Spencer et al. 1991). Because *Mysis* spp. can feed on phytoplankton and zooplankton (Grossnickle 1982), they are not limited by zooplankton availability. Further, because early life stages of the primary piscivores in these systems (kokanee salmon, *Oncorhynchus nerka*) rely on zooplankton, *Mysis* spp. can negatively affect their eventual predators through exploitative competition. Because *Mysis* spp. vertically migrate (Beeton 1960; Spencer et al. 1991), they are often spatially segregated from their visually feeding predators during the day. Thus, in a scenario similar to that of gizzard shad in reservoirs, *Mysis* spp. influence zooplankton abundance via tactile feeding in the upper water column at night, yet enjoy relative immunity from predation in their daytime deep-water refuge.

Conclusions

Our expectation is that through time, reservoirs should move toward one of two steady states, relative to fish community structure. Where gizzard shad are abundant, systems should experience a decline in sport fishes, such as bluegill and largemouth bass, as gizzard shad, through the ecological interactions described above, come to dominance. By exploitatively outcompeting bluegill, gizzard shad also compromise largemouth bass recruitment, reducing predatory pressure on themselves and enhancing their eventual rise to dominance. Under these conditions, gizzard shad in lower latitude reservoirs regulate the community in a weblike fashion, rather than in the chainlike fashion more characteristic of the trophic cascade hypothesis (sensu Power 1992). In reservoir systems without gizzard shad at least occasional strong year-classes of sport fishes will be produced with resultant high-quality angling.

Even though not directly applicable to our systems, the trophic cascade hypothesis has provided an important organizing paradigm. Through comparison, we discovered that a prolific, rapidly growing planktivore with a size refuge and omnivorous diet compromised the strong, chainlike linkages required for a trophic cascade. By exploring mechanistic explanations for food-web structure in reservoirs, as compared with north temperate lakes, we grow to appreciate the diversity of natural systems while simultaneously improving our ability to understand, predict, and manage their complexity.

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Modeling of Visual Selective Attention

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Dr. Joel Weiner, University of Alberta
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Call for Papers

International Symposium on
Assessment and Status of Pacific Rim Salmonid Stocks

North Pacific Anadromous Fish Commission
October 28-29, 1996
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The North Pacific Anadromous Fish Commission (NPAFC) announces an international symposium on assessment and status of Pacific Rim salmonid stocks, to be held October 28-29, 1996, at the Hokkaido University Conference Hall in Sapporo, Japan. The symposium will be hosted by the Government of Japan, under the auspices of the NPAFC and organized by the steering committee of the NPAFC. The local arrangements are being made by a local organizing committee established by the host country.

The purposes of the symposium are: 1) to review the status of salmonid stocks in the North Pacific Ocean from the view points of life history, population ecology, and ecosystem, 2) to review methods in monitoring and assessment of stocks, and 3) to review the present knowledge concerning factors affecting salmonid production. Symposium participants will also discuss future research necessary for assessment of salmonid stock conditions in the North Pacific and methodology for cooperative research works.

The oral session will include keynote, invited, and contributed presentations. Each speaker will have 20 minutes for presentation, questions, and discussion.

A poster session will be held in the lobby of the Conference Hall. Presentations will be chosen from abstracts.

The symposium topics will be the following three items.

1. ***Components of Salmonid Life History Concerning Stock Assessment and Status***
2. ***Salmon Population Ecology Affecting Stock Assessment and Status***
3. ***Relation of North Pacific Ecosystem, Climate, and Oceanographic Changes to Salmon Stock Production***

WORKING LANGUAGE
The language for the symposium is English.

ABSTRACTS
All participants will be invited to submit abstracts for either oral or poster presentation. Abstracts should be received by the end of May 1996, at the NPAFC Secretariat. The Steering Committee will select presentors. Details (or guidelines) for presentation and submission of papers will be sent to presentors by late June.

PUBLICATION
Proceedings of the symposium for both oral and poster presentations will be published by the NPAFC. Manuscripts of scientific papers should be received at the symposium. The manuscripts will be sent out for peer technical review, and the authors will be given the opportunity to make revisions before publication.

INFORMATION
For more information, please contact Mr. Hisashi Endo, NPAFC Secretariat, 6640 Northwest Marine Drive, Vancouver, B.C. V6T 1X2, CANADA. Phone: (604)228-1128, Facsimile: (604)228-1135, e-mail: endo@unixg.ubc.ca.

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Thomas A. Johnston, Marc N. Gaboury, Richard A. Janusz, and Laureen R. Janusz	**2423–2431**	Larval fish drift in the Valley River, Manitoba: influence of abiotic and biotic factors, and relationships with future year-class strengths
Steven J. Hamilton and Terry A. Haines	**2432–2444**	Influence of fluoride on aluminum toxicity to Atlantic salmon (*Salmo salar*)
S.E. Campana, R.K. Mohn, S.J. Smith, and G.A. Chouinard	**2445–2456**	Spatial implications of a temperature-based growth model for Atlantic cod (*Gadus morhua*) off the eastern coast of Canada
John C. Bonardelli and John H. Himmelman	**2457–2469**	Examination of assumptions critical to body component indices: application to the giant scallop *Placopecten magellanicus*
Richard C. Back, Vanessa Visman, and Carl J. Watras	**2470–2475**	Microhomogenization of individual zooplankton species improves mercury and methylmercury determinations
Barry A. Berejikian	**2476–2482**	The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator
Shelagh Montgomery, Alfonso Mucci, Marc Lucotte, and Pierre Pichet	**2483–2492**	Total dissolved mercury in the water column of several natural and artificial aquatic systems of Northern Quebec (Canada)
Agnès Grondin, Marc Lucotte, Alfonso Mucci, and Benoit Fortin	**2493–2506**	Mercury and lead profiles and burdens in soils of Quebec (Canada) before and after flooding
Alfonso Mucci, Marc Lucotte, Shelagh Montgomery, Yanick Plourde, Pierre Pichet, and Huu Van Tra	**2507–2517**	Mercury remobilization from flooded soils in a hydroelectric reservoir of northern Quebec, La Grande-2: results of a soil resuspension experiment
		PERSPECTIVE
Roy A. Stein, Dennis R. DeVries, and John M. Dettmers	**2518–2526**	Food-web regulation by a planktivore: exploring the generality of the trophic cascade hypothesis

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Volume 52, numéro 11, novembre 1995

ARTICLES

John B. Taggart, Eric Verspoor, Paul T. Galvin, Paloma Morán, and Andrew Ferguson	**2305–2311**	A minisatellite DNA marker for discriminating between European and North American Atlantic salmon (*Salmo salar*)
David H. Wahl	**2312–2319**	Effect of habitat selection and behavior on vulnerability to predation of introduced fish
D.G. Worthington, A.J. Fowler, and P.J. Doherty	**2320–2326**	Determining the most efficient method of age determination for estimating the age structure of a fish population
Martha E. Mather, Michael J. Vanni, Thomas E. Wissing, Scott A. Davis, and Maynard H. Schaus	**2327–2338**	Regeneration of nitrogen and phosphorus by bluegill and gizzard shad: effect of feeding history
J.O. Sunyer, E. Gómez, V. Navarro, J. Quesada, and L. Tort	**2339–2346**	Physiological responses and depression of humoral components of the immune system in gilthead sea bream (*Sparus aurata*) following daily acute stress
Karin C. Hårding and Tero Härkönen	**2347–2352**	Estimating mean age at sexual maturity in the crabeater seal (*Lobodon carcinophagus*)
C. Braxton Dew	**2353–2366**	The nonrandom size distribution and size-selective transport of age-0 Atlantic tomcod (*Microgadus tomcod*) in the lower Hudson River estuary
G. Köck, R. Hofer, and S. Wögrath	**2367–2376**	Accumulation of trace metals (Cd, Pb, Cu, Zn) in Arctic char (*Salvelinus alpinus*) from oligotrophic Alpine lakes: relation to alkalinity
E.M. DeBlois and G.A. Rose	**2377–2387**	Effect of foraging activity on the shoal structure of cod (*Gadus morhua*)
Euan D. Reavie, John P. Smol, and N. Bruce Carmichael	**2388–2401**	Postsettlement eutrophication histories of six British Columbia (Canada) lakes
Matt R. Whiles and J. Bruce Wallace	**2402–2422**	Macroinvertebrate production in a headwater stream during recovery from anthropogenic disturbance and hydrologic extremes

Continued on inside back cover / Suite au verso

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